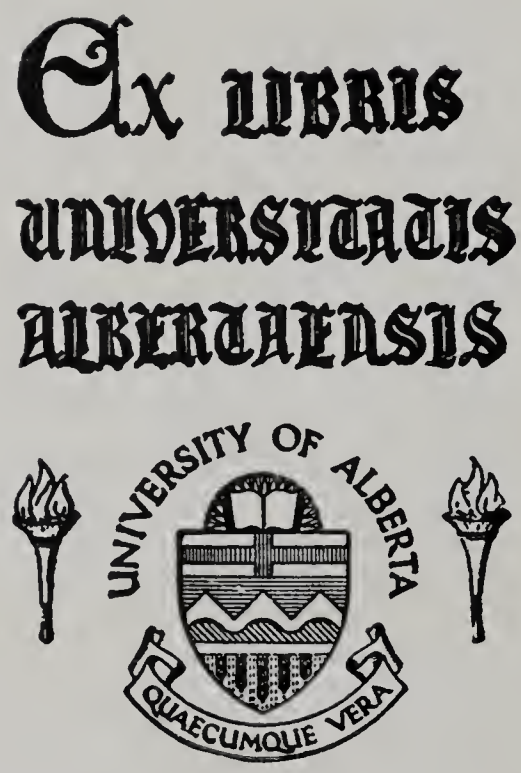

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CALCIUM INCORPORATION AND TRANSLOCATION IN CORN AND WHITE MUSTARD ROOTS

by

PAUL LUKE LEMAY

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES IN

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The undersigned certify that they have read and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled, "CALCIUM INCORPORATION AND TRANSLOCATION IN CORN AND WHITE MUSTARD ROOTS", submitted by Paul Luke Lemay in partial fulfillment of the requirements for the degree of Master of Science.

ABSTRACT

The calcium content of epidermal cell walls and root-hair walls of *Sinapis alba* and *Zea mays* was studied by the radioautographic technique. Radioautographs prepared from strips of epidermis and root cross sections previously treated with Ca^{45} show that calcium is incorporated into the outer layer (pectic layer) of the outer epidermal cell wall and root-hair wall and in the pectic substances cementing the cells together. In mustard roots, treated with Ca^{45} by the moist-air method, the walls and cytoplasm of the short epidermal cells are more radioactive than the walls and cytoplasm of the long epidermal cells. Calcification of the cell walls of a short epidermal cell (hair-forming cell) in mustard, begins with the inner tangential wall directly in line with the accumulation of Ca^{45} in the intercellular spaces. From the inner tangential wall, calcification progresses along the radial walls and then along the transverse end walls. After calcification along the transverse end walls reaches the outer tangential wall, it continues apically over the epidermal cell wall immediately below more rapidly than it does basally over the cell wall above.

Trichoblasts or hair-forming cells form their papillae

at a very early stage in the calcification process of the outer tangential wall. Whether a hair forms or not, the calcification of the outer tangential wall continues from both ends, but more rapidly along the basal end than along the apical end. When a hair is formed from a hair-forming cell, the calcification process is very similar to that described above for the calcification of the outer tangential wall except that when calcification reaches the base of the hair, it continues along the epidermal cell to the adjacent hair walls and then along the walls of the hair to the growing hair tip. The basal convex wall of a young root hair is more strongly calcified than the concave wall of the same hair. In a fully grown hair, the amount of calcium along the wall gradually diminishes from the more strongly calcified base to the only slightly calcified apex, with virtually no calcification over the root-hair dome.

Roots grown in saturated $CaSO_4$ solutions of high concentrations of disodium versenate, low pH, or medium concentrations of boric acid showed signs of calcium deficiency. Roots grown in saturated $CaSO_4$ solutions of low concentrations of disodium versenate, moderately high pH, and no boric acid, showed signs of over calcification.

Roots grown in contact with Ca^{45} and the labelled areas assayed by a Geiger Mueller counter show that radioactivity increases from a minimum in the root cap to a maximum in the mature region.

ACKNOWLEDGEMENTS

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INTRODUCTION

Calcium pectate is generally considered to be the chief structural substance cementing together the walls of adjacent plant cells. Currently, because of its stiffening action when combined with pectic materials, considerable attention is being given to the role of calcium in cell-wall growth.

The previous portion of this study (Cormack, Lemay and Maclachlan, 1962) was concerned with the distribution of Ca^{45} in the root-hair walls of white mustard, corn and tomato seedlings. The walls of growing root hairs developed in moist air following treatment with $Ca^{45}Cl_2$ were tested for incorporated Ca^{45}. Radioautographs show that calcification of the wall in actively growing hairs begins just back of the growing tip and increases towards the base of the hair. Further experience in the handling of radioactive isotopes was gained in a subsequent study designed to determine the presence of glucose-1-C^{14} in the intercellular space of white mustard roots (Cormack and Lemay, 1963).

This thesis extends the investigations with $Ca^{45}Cl_2$. Initially, interest was limited to refinements of technique by which the incorporation of Ca^{45} into the elongating epidermal cell wall and the root-hair wall could be detected at a very early stage. It was also hoped that these refinements of technique would make it possible to distinguish that part of the wall where a hair originates from other

parts of the same cell wall, and to distinguish between a hair-forming cell and a hairless cell.

Although the view that calcium pectate is present in the root-cell wall has been confirmed by numerous workers, the function of calcium as a stiffening substance involved in cell wall growth has been supported (Roberts, 1916; Cormack, 1935, 1944, 1945, 1949, 1954, 1955, 1956, 1959a, 1959b, 1961; Tagawa and Bonner, 1957; Cooil and Bonner, 1957; Brewbacker and Kwack, 1963) and disputed (Ekdahl, 1948, 1953, 1957a, 1957b; Belford and Preston, 1961). It was hoped that the combined results of growing seedlings in control solutions of calcium, and the radioautographic technique, would give an insight into the function of calcium pectate as a cell wall growth substance.

The translocation pathways of calcium to its points of deposition or incorporation in the corn and mustard root is poorly described in textbooks. Much interest was aroused in this study in the translocation of calcium, not only because more work was needed in this field, but also because a better understanding of its translocation in the root would throw light on its distribution and incorporation.

LITERATURE REVIEW

Since its discovery in 1665 by Robert Hooke, the plant cell wall has been the object of uninterrupted investigation. In the intervening three hundred years a great deal has been learned concerning its structure and chemical composition which are the key to an understanding of its growth and development. Recent reviews and detailed discussions include those by Frey-Wyssling (1957), Northcote (1958), Preston (1958), Roelofsen (1959), Setterfield and Bayley (1961), Wardrop (1962) and Cormack (1949, 1962), while recent papers dealing with specific aspects of cell-wall composition and growth are all too numerous to mention.

The present literature review deals almost exclusively with recent papers on calcium in the primary cell wall and root-hair wall, its effect on cell-wall stiffening and the translocation of calcium.

Roberts (1916), experimenting with root-hair walls of numerous crop plants, recognized that in a trichoblast the epidermal cell wall and the root-hair wall are continuous. Since this early study, it has been accepted that the walls are not only continuous but are also similar in structure and chemical composition. For this reason studies on the epidermal cell wall and the root-hair wall will be discussed together.

Cellulose and pectic substances have generally been

thought to be the chief structural materials in the primary wall, while the intercellular substance (middle lamella) that cements the walls of adjacent cells is considered to be composed of insoluble salts of pectic or low-methoxyl pectinic acids.

Information concerning chemical changes in growing cell walls has been obtained through studies on micro-chemical testing, maceration by means of enzymes (Bryan and Newcombe, 1954; Glaziou, 1959; Cormack, 1955, 1956; Zaitlin and Coltrin, 1964) and calcium chelating reagents (Cormack, 1935, 1959; Heath and Clark, 1956) and on the effects of various culture solutions (Goodwin and Avers, 1956; Burström, 1952; Cormack, 1956). The results of these investigations show that stiffening of the cell wall is an important factor in cell-wall elongation. The mechanism by which hardening is affected is not clearly understood and some of the proposals put forward to explain this process are in sharp disagreement.

I. Theories of Epidermal Cell Wall and Root-hair Wall Stiffening

A. Cellulose Controlling Cell-Wall Rigidity

Earlier ideas of cell-wall stiffening were centered around the cellulose framework of the plant cell wall. Many authors believed that the amount of cellulose microfibrils or the rate of

microfibril deposition determines the rate of cell wall stiffening or rigidity (Adamson and Adamson, 1959; Bennet-Clark, 1961; Ekdahl, 1953; Frey-Wyssling, 1952; Galston and Purves, 1960; Ray, 1961; Setterfield and Bayley, 1961; Thimann, 1954). The actual process involved in cell-wall elongation has been variously described by the above authors with the use of such terms as re-orientation of fibers (Frey-Wyssling, 1952), partial dissolution (Thimann, 1954), loosening (Ray, 1961), relaxing (Galston and Purves, 1960), softening (Bennet-Clark, 1961) increased wall flexibility (Adamson and Adamson, 1959), of the cellulose framework. Elongation, in the opinion of these authors, is a phenomena controlled by the cellulose portion of the wall.

The literature on the cellulose theories of cell-wall stiffening has been well reviewed (Cormack, 1949, 1962; Belford and Preston, 1961; Ekdahl, 1953; Young, 1962; Wardrop, 1962) and will not be reviewed further here.

More recent experiments on cell walls show that factors favoring cellulose deposition (Young, 1962), still render the wall plastic with little mechanical strength. Elongation has been reported to occur with a slight drop in the total amount of cellulose

(Solberg and Higinbotham, 1957). These findings have led researchers to look at wall elongation from different points of view.

B. "Pectin Theory" of Wall Stiffening

Quantitative chemical studies of the cell-wall components by Setterfield and Bayley (1961) measured in different species, have been recorded as cellulose 20-50%, hemicellulose 4-5%, pectin 1-50%, protein 2-30%, lipid 1-25%.

The pectic component is known to go through a series of chemical reactions through which there is a gradual stiffening of cell walls. In non-lignified tissues, the strongest of these cementing substances is calcium pectate (Roberts, 1916; McCoy, 1932; Farr, 1925, 1927, 1928; Cormack, 1935, 1944, 1945, 1949, 1954, 1955, 1956, 1959a, 1959b, 1961; Cleland and Bonner, 1956; Cooil and Bonner, 1957; Ordin and Bonner, 1957; Tagawa and Bonner, 1957; Deuel and Stutz, 1958; McClendon and Somers, 1960; Weintraub and Ragetli, 1961; Zaitlin and Coltrin, 1964).

The more recent work on the growth of root hairs and pollen tubes has strongly supported the "Pectin Theory" (Cormack and Lemay, 1963; Brewbacker and Kwack, 1963). In these studies it has been shown that small amounts of calcium are necessary for

elongation, while over-calcification results in excessive stiffening of the cell wall and consequently the retardation or inhibition of growth.

The "Pectin theory" has also been criticized by numerous workers. Bishop et al. (1958) state that the pectin content of the wall is too small to give the cell wall the stiffening it receives under certain conditions. Carr and Ng (1959) claim that the calcium content of calcified walls is not plentiful enough to afford the wall stiffening that is apparent in certain experiments. Setterfield and Bayley (1961) are very skeptical of their own findings, and state that the theory of pectic substances is attrative but unsubstantiated.

It is clear that the problem of cell-wall stiffening is far from being well understood. More research in this field is necessary.

II. Translocation of Calcium

As early as the turn of the last century, it has been intimated or even stated from the results of deficiency studies (True, 1914) or direct concentration studies (True, 1922) that mineral ions must be absorbed or translocated through the plant root.

Deficiency and concentration studies, however, do not allow the pathway of translocation to be followed.

Only the end result is observed from which it may be concluded that translocation has occurred or not.

In an attempt to trace the translocation pathway of water and mineral ions from the solution medium to the shoot and leaves, Strugger (1937-1938, 1939), has added acidic and basic dyes to the solution media. His investigation has shown that substances could be drawn by the transpiration stream along cell walls to the stele. Because of the small amount of cytoplasm around the wall, in older cells of roots it was not possible to establish whether the dyes remained outside the plasma membrane or whether they entered the plasmalema and were retained out of the tonoplast. It was clear that there was no dye in the vacuole.

More recent investigations (Crafts, 1949, 1961; Broyer, 1950) made use of radioactive ions in an effort to localize the transport pathway. Broyer (1950) has shown that they pass from the culture medium to the xylem without being accumulated in vacuoles.

Details of ion transport from the culture media to shoot are poorly established and very controversial.

Since there are recent literature reviews on the problem of ion transport (Broyer and Stout, 1959; Epstein, 1956; Hylmö, 1953; Lundegårdh, 1955) it does not merit repeating here.

The literature reviews clearly show that little is

known of ion transport in general and still less of calcium transport in particular. To understand the complete translocation pathway of any one ion, it is necessary to trace it from its point of entry along whatever pathways it takes to areas of distribution and incorporation. Specific findings on translocation of calcium would augment the present more general translocation pathways described by such investigators as Crafts, A.S. (1949, 1961); Biddulph, O. (1958); Biddulph, S. (1958); Cory, R. (1958); Epstein, E. (1956, 1961); and Koontz, H. (1958).

MATERIALS AND METHODS

MATERIALS

Seedlings of *Sinapis alba* L. were used as the experimental material because the seeds germinate readily, and the roots develop abundant root hairs in water. Also considerable anatomical work has already been done with this particular plant (Cormack, 1935, 1947, 1948; Belford and Preston, 1961; Cormack, Lemay and Maclachlan, 1962; Cormack and Lemay, 1963). Later, confirmatory tests were carried out with *Zea mays* L. seedlings because of the prominent endosperm which facilitates the introduction of experimental material. White mustard (*Sinapis alba* L.) and corn (*Zea mays* L.) were obtained from Steele-Briggs Seed Company, Edmonton.

$Ca^{45}Cl_2$ (0.8 mc/ml) which was used as the tracer material was obtained from the Atomic Energy of Canada Ltd.

METHODS

Germination of Mustard and Corn Seeds

Seeds of white mustard and corn were first soaked in a .001M solution of mercuric chloride for three minutes and five minutes, respectively, to kill any fungus spores. The seeds were then rinsed in tap water and placed in a stream

of running tap water to soften the seed coats. Water was floated over the mustard seeds for one hour and over the corn seeds for twelve hours. At the end of the soaking period, the seeds showed variability in the amount of water imbibed, ranging from no noticeable signs of swelling to extreme swelling. Moderately swollen seeds were removed and placed in petri dishes between layers of filter paper moistened with demineralized distilled water. The petri dishes were then placed in an incubator at 23°C up to the time that the radicle began to break through the seed coat. Seedlings with roots ranging between first signs of emergence, and two millimeters in length, were considered ready for treatment with labelled calcium.

I. Cell-Wall Studies

A. The Introduction of Radioactive Calcium

1. Moist-Air Method

The general procedure was to place the seedlings in petri dishes in contact with filter paper well soaked with 10^{-2}M $Ca^{45}Cl_2$ solution.

At five-hour intervals several seedlings were removed, the roots severed and subsequently tested by several methods to determine the translocation and incorporation of labelled calcium.

2. Root-Tip Immersion

A trough was prepared by placing two glass

slides one-half of an inch apart in a microscope slide box. Paraffin was poured on the outside of the two slides, leaving an area in the center free of paraffin. When the paraffin hardened, the slides were removed and a little paraffin was placed in the trough to seal the bottom. Filter paper moistened with demineralized distilled water was placed over the paraffin and fitted into the trough. The germinating seeds were placed on the edge of the trough in such a way that the radicles grew into the trough in contact with the moistened filter paper (Figure 1). When the roots had reached a length of one centimeter, enough $Ca^{45}Cl_2$ solution was syringed into the trough to bring the level of the solution sufficiently high to cover the tips of the roots.

3. The Introduction of Calcium Through the Cotyledons

Because of the large endosperm in corn seed and the absence of endosperm in mustard seed, the procedure of introducing the calcium was different in each case.

(a) Method For Mustard (Ring Method)

Small glass rings were filled with paraffin to within two millimeters of the tip. A

piece of filter paper the same size as the ring was then placed over the paraffin. The filter paper was then well moistened with the 10^{-2}M $Ca^{45}Cl_2$ solution. When the seedlings were ready for treatment, a portion of the cotyledons was removed by means of a sharp razor blade, in such a way as not to injure the embryo. A portion of the seed coat and cotyledons was removed from each seedling opposite the point of root protrusion. Each seedling was placed in the ring so that the cut surfaces of the cotyledons were in direct contact with the moistened filter paper, Figure 2 (Cormack and Lemay, 1963).

(b) Method For Corn (Hole bored into the endosperm)

In order to supply the seedling with radioactive calcium, a small hole was carved in the endosperm of the corn seed (Figure 3). In one set of experiments, this hole was filled with a 10^{-2}M $Ca^{45}Cl_2$ solution. In a second set of experiments, this hole was filled with 10^{-2}M $Ca^{45}Cl_2$ and 10^{-6}M boric acid. The treated seeds were placed on moist filter paper in a petri dish, for further growth.

FIGURE 1 (X1) Diagram of the apparatus used in root-tip immersion. The front of the slide box has been cut away to show the structure of the trough covered by filter paper, and the position of the seed at a higher level.

FIGURE 2 Diagram of the apparatus used in the ring method of introducing Ca^{45} through the cut cotyledons of white mustard. The mustard seedling was drawn with the use of a camera lucida at a magnification of X6.

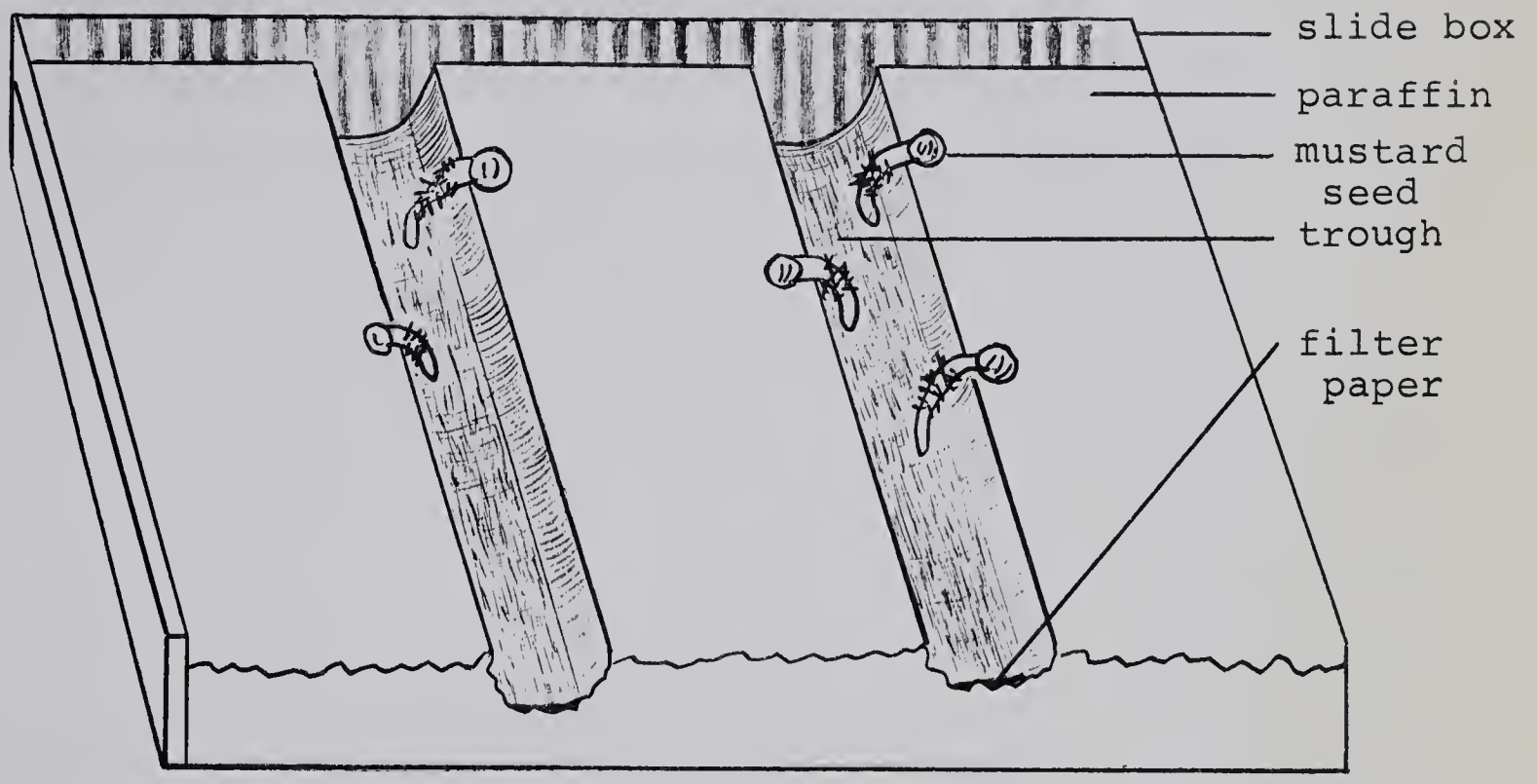


FIGURE 1

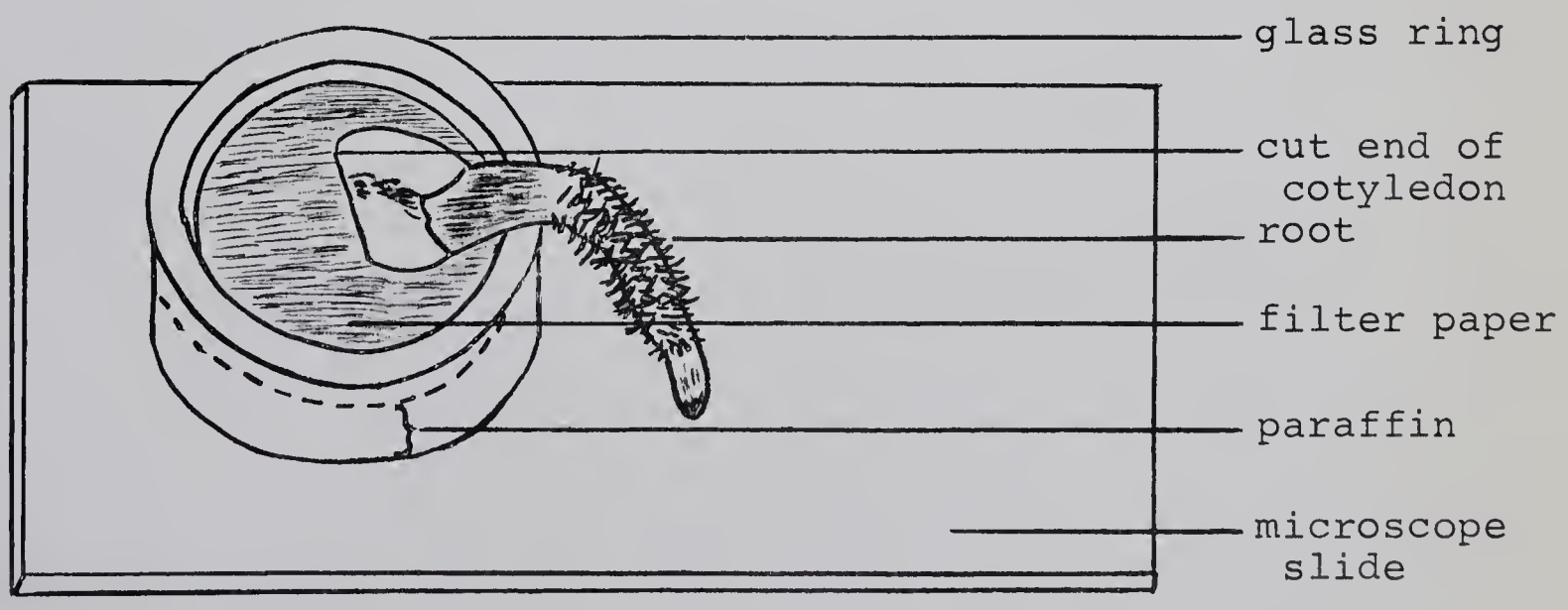


FIGURE 2

FIGURE 3 (X1) Diagram of a corn root grown on a moistened filter paper in a petri dish. Chemicals are introduced through the hole carved in the endosperm.

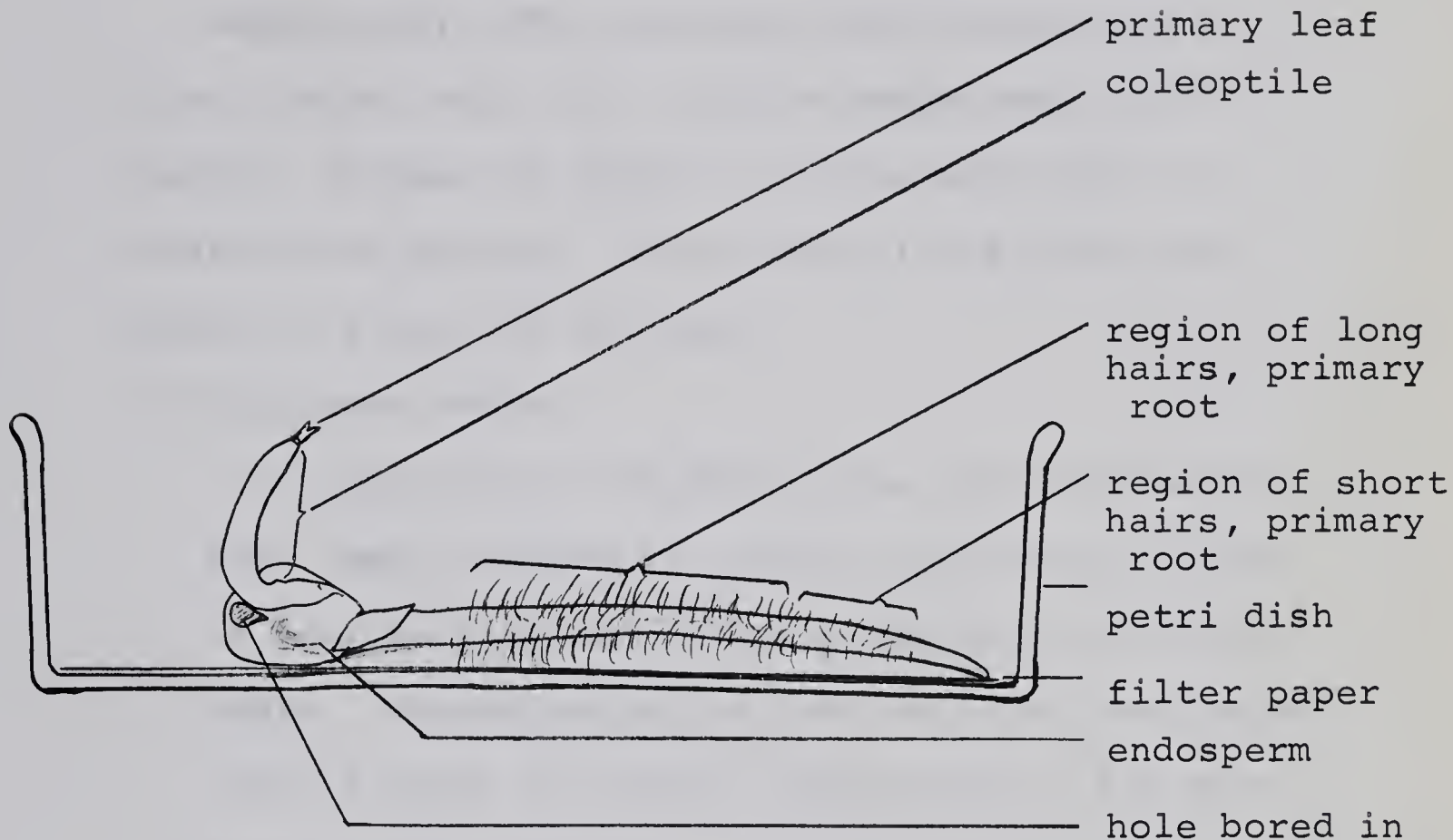

primary leaf
coleoptile
region of long hairs, primary root
region of short hairs, primary root
petri dish
filter paper
endosperm
hole bored in endosperm

FIGURE 3

B. Radioautographic Procedure of Examining the Introduced Radioactive Calcium

Immediately after removing the test material from contact with Ca^{45}, it was washed well with several changes of $CaCl_2$ to remove any excess of radioactive calcium. The material was then prepared by either of two ways.

1. Microtome Method

In preparation for sectioning with the microtome, small pieces of treated roots were placed in Craf killing and fixing reagent, for fifteen hours. Subsequently the root material was washed under running tap water, dehydrated in the usual manner with n-butanol, stained with erythrosin B, infiltrated and embedded in tissuemat. The pieces in absolute n-butanol were poured over an equal amount of 50/50 paraffin and paraffin oil and placed in an oven at 58°C. When all of the absolute n-butanol had evaporated, the 50/50 paraffin and paraffin oil were poured off and replaced by paraffin, which was left twelve hours. Three changes of tissuemat followed. Before pouring the last tissuemat into a paper boat to harden, the oven was turned up to 60°C to facilitate pouring. The tissuemat was quickly poured into little paper boats (2 x 4 cm.) on a

warming plate which kept the tissuemat from hardening too quickly, and thus the roots could be arranged as desired, with warm needles. Then the tissuemat was quickly cooled by submerging the boats in ice water. The roots were cut from the blocks, and trimmed with a razor blade in preparation for sectioning.

2. Stripping Method

Severed roots were placed in boiling water for two hours. Following heating, the roots were placed in water on a microscope slide and fine strips of epidermis were removed by means of sharp needles. A satisfactory strip of epidermis was selected and floated in a drop of water on another slide previously coated with Haupt's adhesive, and further teased apart by fine needles.

C. Steps in Slide Preparation

1. Adhering Material to Slides

(a) Microtome Method

Both transverse and longitudinal sections were cut at 10 microns on a rotary microtome (American Optical Company Model 815). Sections of ribbons were floated out on a Haupt's coated slide with a 5% formalin solution. Slides were then heated to 50°C for two hours to flatten the ribbons and

adhere the ribbons to the slides.

(b) Stripping Method

The strips of teased material were placed on a slide previously coated with Haupt's solution, and dried.

D. Coating of Slides with Emulsion, and Exposure

The light tight emulsion bottle was opened and the G-5 emulsion was handled in the dark room with the use of light screened by a P-5 filter. The G-5 emulsion (melting point 48°C) was heated to 50°C and a small quantity of demineralized distilled water was added to produce a thinner coat on the slide. The dried slides bearing either thin sections or strips of epidermis were tilted into the emulsion in such a manner that the root tissue was uniformly covered. The slides were then dried under a stream of warm air provided by a hair-drying fan, placed in dark light-tight boxes containing a desiccant ($CaCO_3$) and transferred to a refrigerator at 4°C for exposure.

E. Developing Emulsion-coated Slides

Slides were developed in D-19 developer, diluted 1:3 with distilled water, for ten minutes. Slides were moved to 3% acetic acid stop bath for three minutes, 30% hypo for nine minutes, and tap water for twenty minutes.

The slides were dried. Sections were covered with glycerine and a cover glass, with sides sealed with Permount.

F. Photoradioautographs

The prepared sections and strips of epidermis were observed with a Zeiss Photomicroscope (55575). Photomicrographs were taken on Adox KB 14 film (ASA 20). Films were developed at 18°C in Perinal diluted 1:8, placed in 3% acetic acid, and fixed in acid fixer (Eastman Kodak Company). The film was then washed for a half hour in running tap water controlled at 18°C, dipped in Photo-flo (Eastman Kodak Company), and hung to dry.

Prints were made using a Beseler Enlarger, Model 45MX, on Kodabromide single weight F-4 paper, and developed with Dectol diluted 1:1.

G. Non-Radioactive Calcium Culture Solutions

Seeds of mustard and corn were germinated as mentioned above. Germinated seeds of average size were placed on paraffin-coated paper floats that had small holes pierced through them to allow the the roots to grow down into the solution.

1. EDTA Solutions

A series of ten different EDTA concentrations were prepared by adding quantities of EDTA (saturated solutions at room temperature) to

200 c.c. of a solution of $CaSO_4$ 10^{-2}M with .2 x 10^{-2}M boric acid to study the effects of calcium concentration on the root cell walls.

2. Solutions of Different pH Concentrations

A series of ten tris buffered pH concentrations from 2 to 13 were made up with $CaSO_4$ 10^{-2}M to study the effect of pH on calcium incorporation.

3. Boric Acid Solutions

A series of ten boric acid concentrations were made up (0 to .2M) and placed in a constant calcium, pH 8.2 mixture for corn and pH 7.4 for mustard to study the effect of boric acid on calcium incorporation and translocation.

II. Translocation and Distribution Studies Using Ca^{45}

A. Radioautographic Technique

The radioautographic technique was identical to that already described. When the microtome method is used, serial sections are carefully prepared so that distances from the tip may be calculated.

B. Quantitative Test For Calcium Distribution By Means of a Geiger Counter

Seedlings were prepared as in section I-A (The Introduction of Radioactive Calcium). The seedlings were well washed with several changes

of $CaCl_2$. Next the roots were severed from the seedlings and each root cut into short pieces of a specific length beginning at the apical end. In some of the roots, the stele was separated from the cortex of each small piece by means of a fine pair of needles. Each piece in series was then removed by forceps and placed on a numbered piece of glossy paper, then dried in an oven at 40°C. Following drying, each piece was weighed individually on a Gramatic Balance, crushed in a test tube mortar with a glass rod pestle, covered with demineralized distilled water, filtered on to a glass fiber paper (No. 934-AH), dried on a planchet, and the radioactivity counted on a Geiger counter (Nuclear Chicago Model 186).

RESULTS

EXPERIMENTS WITH Sinapis alba AND Zea mays

I. Cell Wall Studies

A. Radioactive Calcium

Before presenting the results obtained by radioautography, some explanation of terminology is necessary.

Radioautograph:	A record of radioactivity shown by the darkening of grains of silver in the emulsion covering the labelled material.
Photoradioautograph:	A photograph of a radioautograph taken at one point of focus of the microscope at one level in the emulsion.

Although the photoradioautograph shows whether radiation has occurred or not, it only records the radioactivity at one depth of focus and over a limited portion of the microscopic

field. The photoradioautographs presented in this thesis will supplement the observations made by focussing up and down with the microscope on the radioautograph itself. It should also be borne in mind that all blackening shown in a photoradioautograph is not due to radiation. An area darkened by radiation can be distinguished from a non-radioactive darkened area by a conspicuous granular or specked outer border.

1. Epidermal Cell Studies

In this thesis, the experiments with corn and mustard roots can be described best by photographic enlargements of radioautographs. A special feature of mustard roots is the differentiation of the epidermis into alternating rows of short and long cells, (18, 24). Normally the short cells produce the hairs, while the long cells remain hairless. The epidermis of corn roots is not differentiated. All the cells are alike and any cell may form a root hair.

Figure 4 shows a row of three epidermal cells as seen in three-dimensional view at the stage of early root-hair formation and should be referred to for an understanding

FIGURE 4 Diagram of an epidermal cell and its root hair, with the surrounding cells in three dimensional view. The terms used in the text are labelled on the diagram.

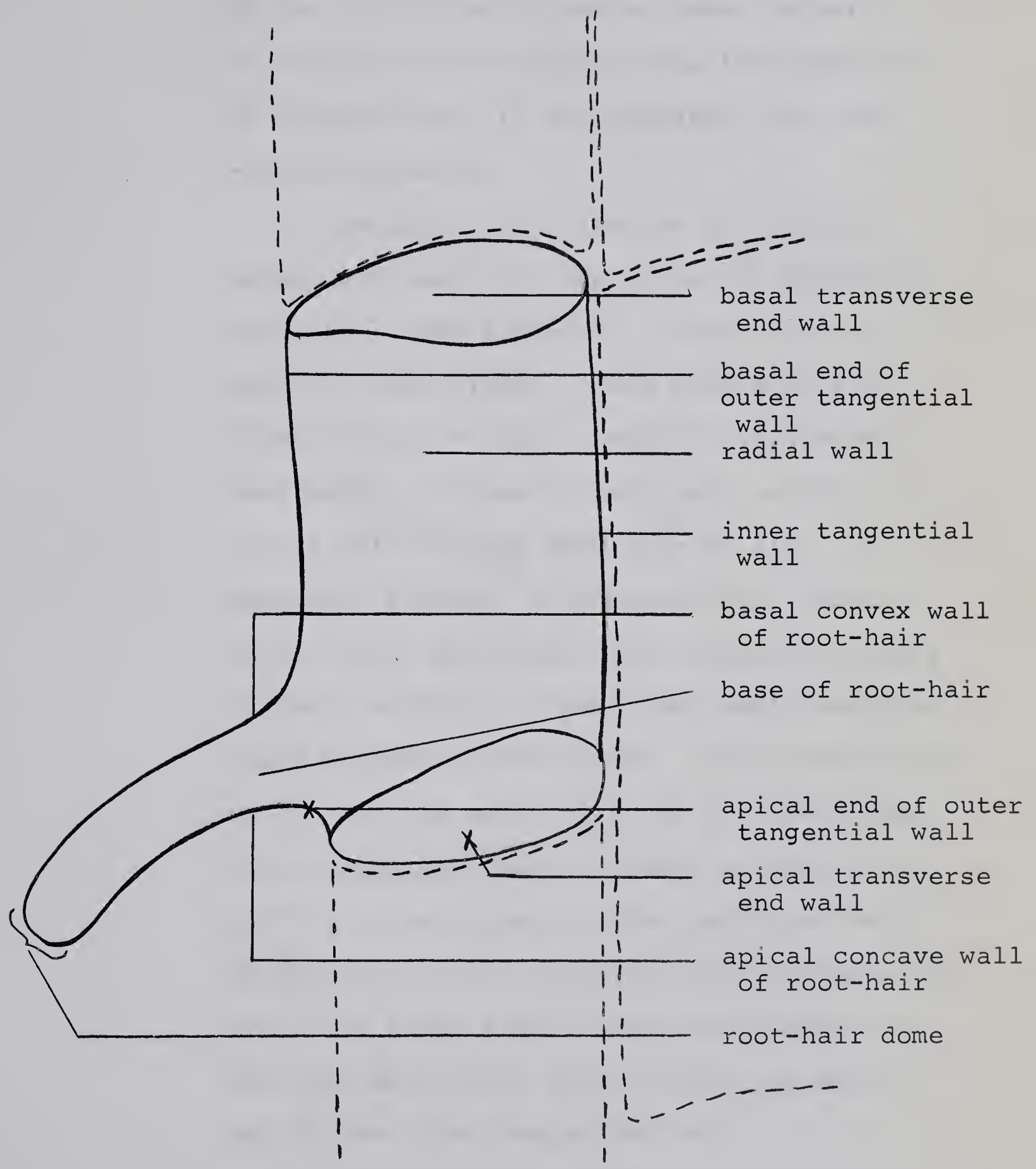

basal transverse end wall
basal end of outer tangential wall
radial wall
inner tangential wall
basal convex wall of root-hair
base of root-hair
apical end of outer tangential wall
apical transverse end wall
apical concave wall of root-hair
root-hair dome

FIGURE 4

of the various walls and surfaces referred to in the text to describe the localization of radioactivity in the epidermal and root -hair cell walls.

In general, calcification of the epidermal cell walls in the region of root-hair formation, some 1500-2500 μ from the root apex, is very slight. Some pieces of epidermal tissue at this stage of development show merely a trace of cell wall calcification while others show none at all. As mentioned already, a characteristic feature of the short epidermal cells of mustard roots is their ability to "push out" small papillae which become the root hairs. The distribution of Ca^{45} in the walls of a row of plasmolyzed short epidermal cells is shown in Figure 5. Ca^{45} is concentrated in the transverse walls of the short cells, slightly less concentrated along the basal end of the outer tangential wall and definitely absent along the apical end of the outer tangential wall.

Although calcium is becoming incorporated into the cell walls of trichoblasts (hair -forming cells) at a slow rate (Fig. 5), it is strongly concentrated in the cytoplasm.

Radioactivity in the cytoplasm appears as a solid blackened area (Fig. 5) but when viewed with the microscope at different points of focus, distinct patterns of radiation can be discerned. These distinct patterns of radiation are such as to suggest that calcium is translocated and possibly incorporated in the endoplasmic reticulum. Even around the nucleus, distinct circles of radiation can be observed.

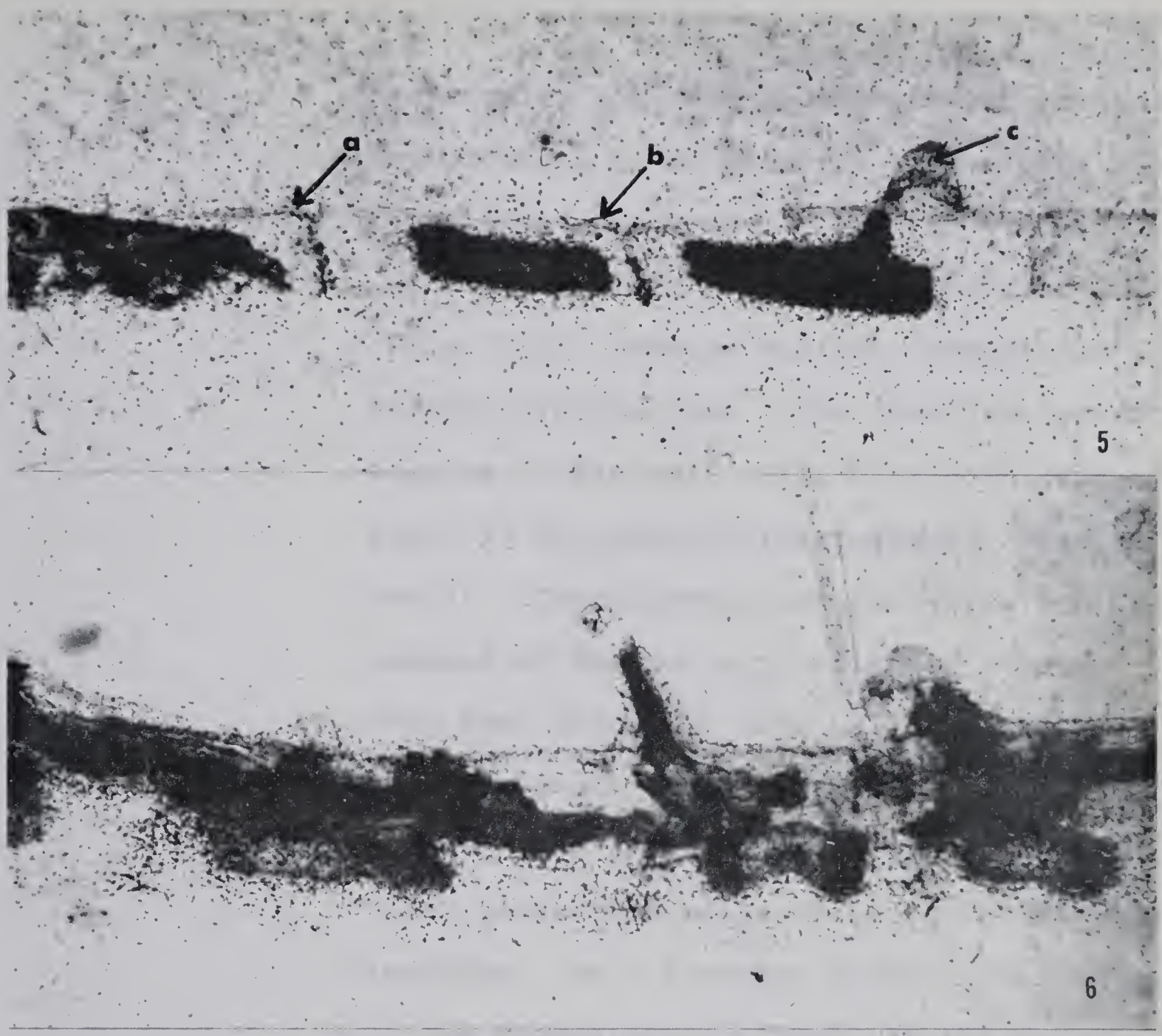
As the epidermal cell matures and the papilla becomes a root hair, calcification procedes at a rapid rate (Fig. 6). In the epidermal cell (Fig. 6) calcium is definitely concentrated in the tangential walls. In the hair itself (Fig. 6) calcium is more concentrated along the basal convex wall than along the apical concave wall. The specific localization of incorporated calcium is even more striking in Figure 7, taken at a somewhat higher magnification. In the longer hair at the right-hand side of Figure 7, the root-hair wall is out of focus, but the cytoplasmic membranes are sharply in focus and show strong radiation. The regular and more densely radiated boundary marking the

Plate I

FIGURE 5 (X325) Photoradioautograph of an epidermal strip with a papilla from a mustard root grown in moist air. Arrows (a) and (b) show the stronger calcification of the basal end of the outer tangential wall. Arrow (c) shows the strands of radiation from the cytoplasm to the papilla wall tip.

FIGURE 6 (X325) Photoradioautograph of an epidermal strip with papillae and short root hairs from a mustard root grown in moist air. Radiation is obvious not only along the outer tangential wall but also along the inner tangential wall. The radiation in the hair is more concentrated along the basal convex than along the apical concave wall.

FIGURE 7 (X810) Photoradioautograph of an epidermal strip from a mustard root grown in moist air. The long slightly plasmolyzed hair has the cytoplasmic boundary well delineated by radiation.



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position of the cytoplasmic membrane suggests that calcium is an integral part of the cytoplasmic membrane.

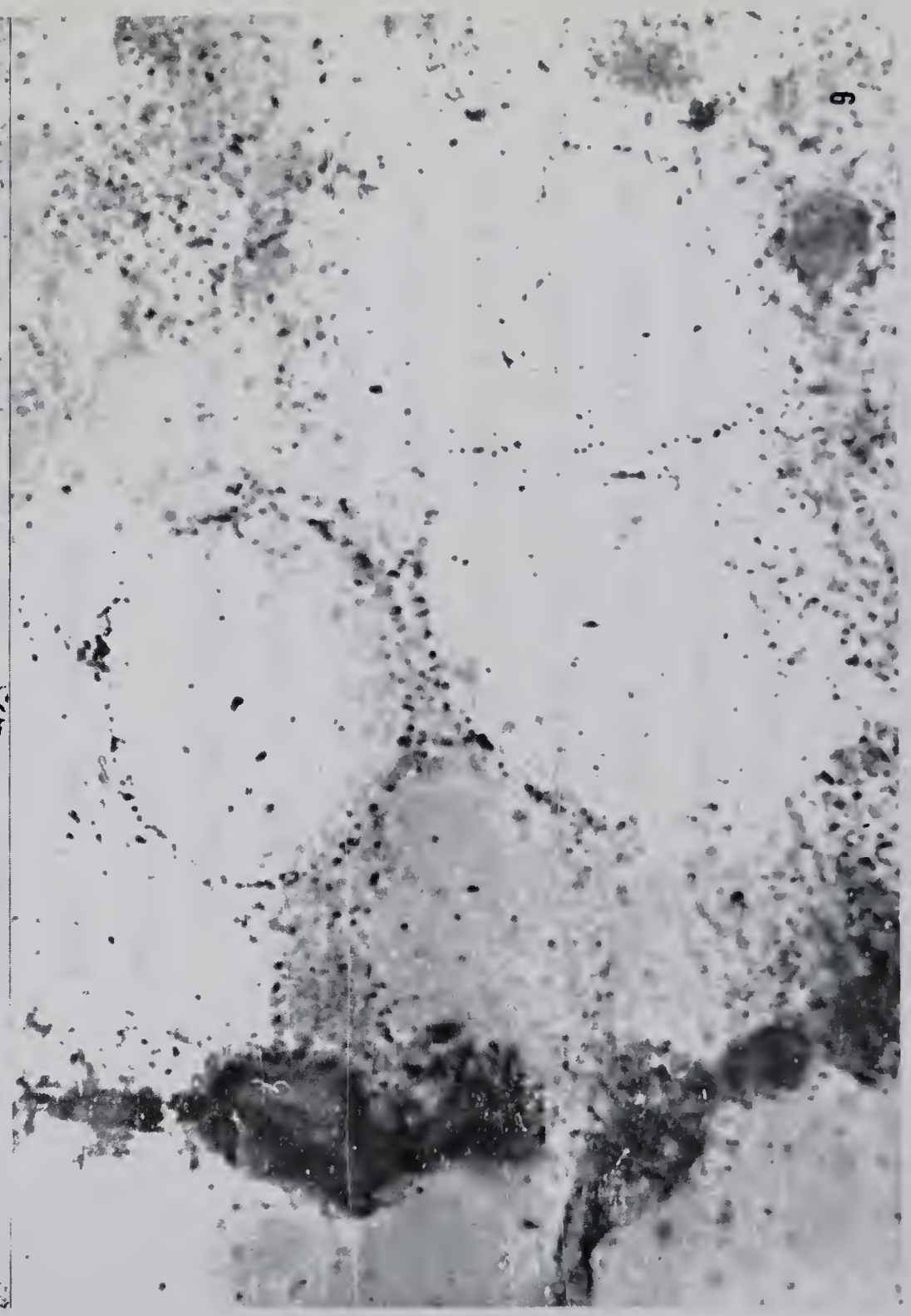
The study of thin transverse sections (Figs. 8-11) reveals similar differences in calcium distribution. The distribution of calcium in the cell walls of a corn root is shown in the photoradioautographs (Figs. 8 and 9). Calcification of the walls at the corners of the cells is slightly greater than that along the side walls, while the outer tangential epidermal cell wall is usually less calcified than the radial and inner tangential walls (Fig. 8) in immature sections. At a somewhat higher level (Figure 9) the accumulation of Ca^{45} in the corners is less distinct. Since these cortical cells have lost their cytoplasm, radioactivity along their walls is exceptionally clear. There is also slight radiation along the tangential and radial walls of the epidermal cells at this same stage (Fig. 9). Another transverse section of a mustard root (Fig. 10) shows that calcium is localized around the vascular bundles and in the epidermis. An exceedingly dark area in the epidermal region represents the position of a short

Plate II

FIGURE 8 (X250) Photoradioautograph of a transverse section of a corn root about 450 μ from the root apex. The cell walls are well indicated by the radiation. Radiation at the corners is more intense than along the side walls.

FIGURE 9 (X810) Photoradioautograph of a transverse portion of the epidermis and two rows of cortical cells of a corn root. The radiation is from the cell walls. The hair extending from the epidermis is poorly focussed.


8


9

hair-producing cell and the light areas between two such dark areas represents the position of hairless long cells. Figure 11, taken at a higher magnification, shows that the walls of the short hair-producing cells are much more strongly calcified along the radial and inner tangential wall than those of the long cells.

2. Root-hair Studies

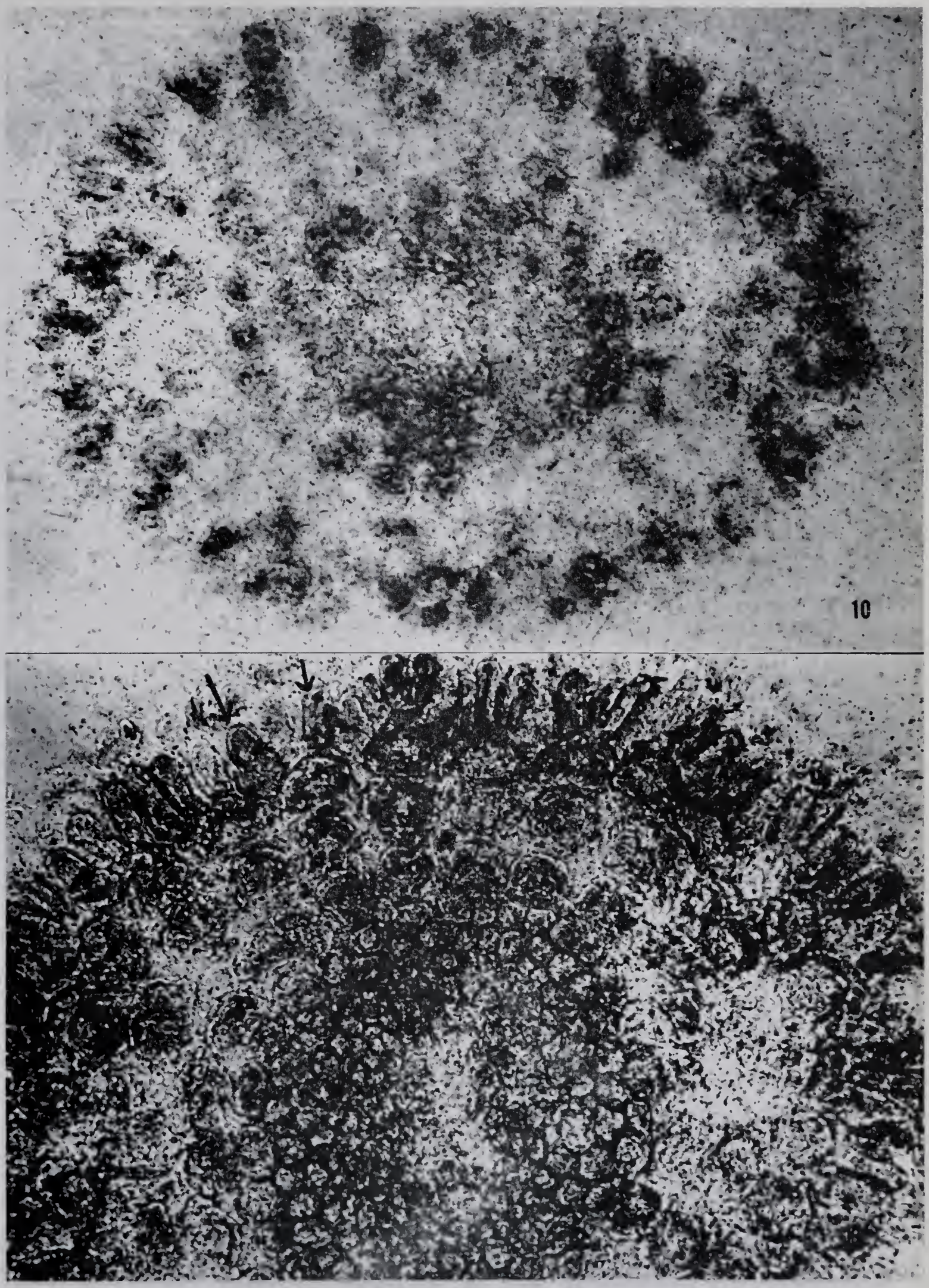
The observations of calcium distribution in the root-hair wall obtained in the present study are in close agreement with those recorded in the earlier work (33).

At the very outset of root-hair development (Fig. 5) differences in the distribution of Ca^{45} in the still elongating cell walls are clearly evident. There is virtually no calcium in the outer tangential wall of the papilla-forming cell or in the wall of the papilla itself (Fig. 5). Although the bulk of the cytoplasm in the papilla itself has drawn well away from the cell wall, the darkened strands of radiation in the papilla tip (arrow (c), Figure 5) could represent delicate cytoplasmic strands still connecting

Plate III

FIGURE 10 (X250) Photoradioautograph of a transverse section of a mustard root at 1020 μ from the root apex. The more intense radiation above the epidermal region represents the short cells. Radiation is also observed in the xylem region of the stele.

FIGURE 11 (X325) Photoradioautograph of a semi -transverse section of a mustard root at 480 μ from the root apex. Radiation above the walls of the short cells is very obvious. The long cells in the epidermis have much less radiation than the short cells.


10

the growing tip of the papilla with the cytoplasm. These cytoplasmic strands are even more apparent in the short hairs shown in Figure 12, (arrow a). In these short actively-growing hairs (Fig. 12), calcification is more intense along the basal convex wall than along the apical concave wall of the hair. Calcification is almost as intense at the base of the hair as it is along the adjacent tangential wall of the epidermal cell itself.

In a much longer hair (Fig. 13), Ca^{45} is distributed throughout the length of the root-hair wall. Radioactivity diminishes gradually from the base of the hair where it is decidedly concentrated, to the very tip where it is very faint or almost absent. Differences in degree of darkening in a photoradioautograph do not always correspond to differences in degree of radioactivity. For instance, the portion of the wall (arrow a) appears lighter than the rest of the hair wall because it is not sharply in focus. On the other hand, the tip of the hair (arrow c) is sharply in focus but appears lighter because of low radioactivity over the

Plate IV

FIGURE 12 (X810) Photoradioautograph of an epidermal strip with a row of cortex still attached, from a mustard root. Radiation along the end walls of the cortical cells is very intense. Also the radiation along the basal convex wall of the hair (arrow b) is more intense than the apical concave wall. Cytoplasmic strands indicated by lines of radiation in the root-hair tip are obvious (arrow a).

FIGURE 13 (X810) Photoradioautograph of a longer plasmolyzed hair of a mustard root. Radiation increases gradually from the root-hair tip (arrow c) to the base of the root hair (arrow b). The root-hair dome has very little radiation. Immediately adjacent to the epidermis, the root hair is out of focus (arrow a).

12

13

rounded tip.

Figure 14 is of a mustard root hair developed on a root whose tip was immersed in Ca^{45}. The results obtained by this method are similar to those obtained when seedling roots are grown on moist filter paper, except that radioactivity is usually more intense and more uniformly distributed along the whole length of the hair. In this particular hair (Fig. 14) the hair has burst at the tip (plasmoptysis). In general, roots grown in a strong Ca^{45} solution show intense radioactivity through-out the root-hair wall and the hairs them-selves are much shorter than those developed on roots grown in less concentrated Ca^{45} solutions. A non-plasmolyzed root hair of white mustard grown in contact with Ca^{45} is shown in Figure 15. Radioactivity in both the cytoplasm and root-hair wall is con-spicuous. The interesting feature of this particular root hair is that radioactivity is much more intense over the rounded dome of the root-hair tip than that shown along the side walls. There is no doubt as to the presence of Ca^{45} in the rounded dome of

the root-hair tip (Fig. 15) but there is some evidence (92) to show that here calcium forms a complex with secretions other than pectic substances and that the wall at the tip is weaker than if it consisted of calcium pectate. The presence of weaker cell-wall materials at the very tip of the root hair could explain the continued growth of the hair at this point.

The distribution of Ca^{45} along the root hair and epidermal cell walls of a corn root grown in moist air (Fig. 16) is almost identical to that observed in mustard roots grown under the same conditions. However, in corn, the difference in the amount of radiation at the opposite ends of the outer tangential wall of a single epidermal cell is not nearly so well defined as it is in mustard roots.

II. Translocation and Distribution Studies by the Radioautographic Technique, and Culture Solutions

A. Radioautographic Technique

The results of this series of experiments show that Ca^{45} is translocated through the root tip when the root tip is immersed in the Ca^{45}

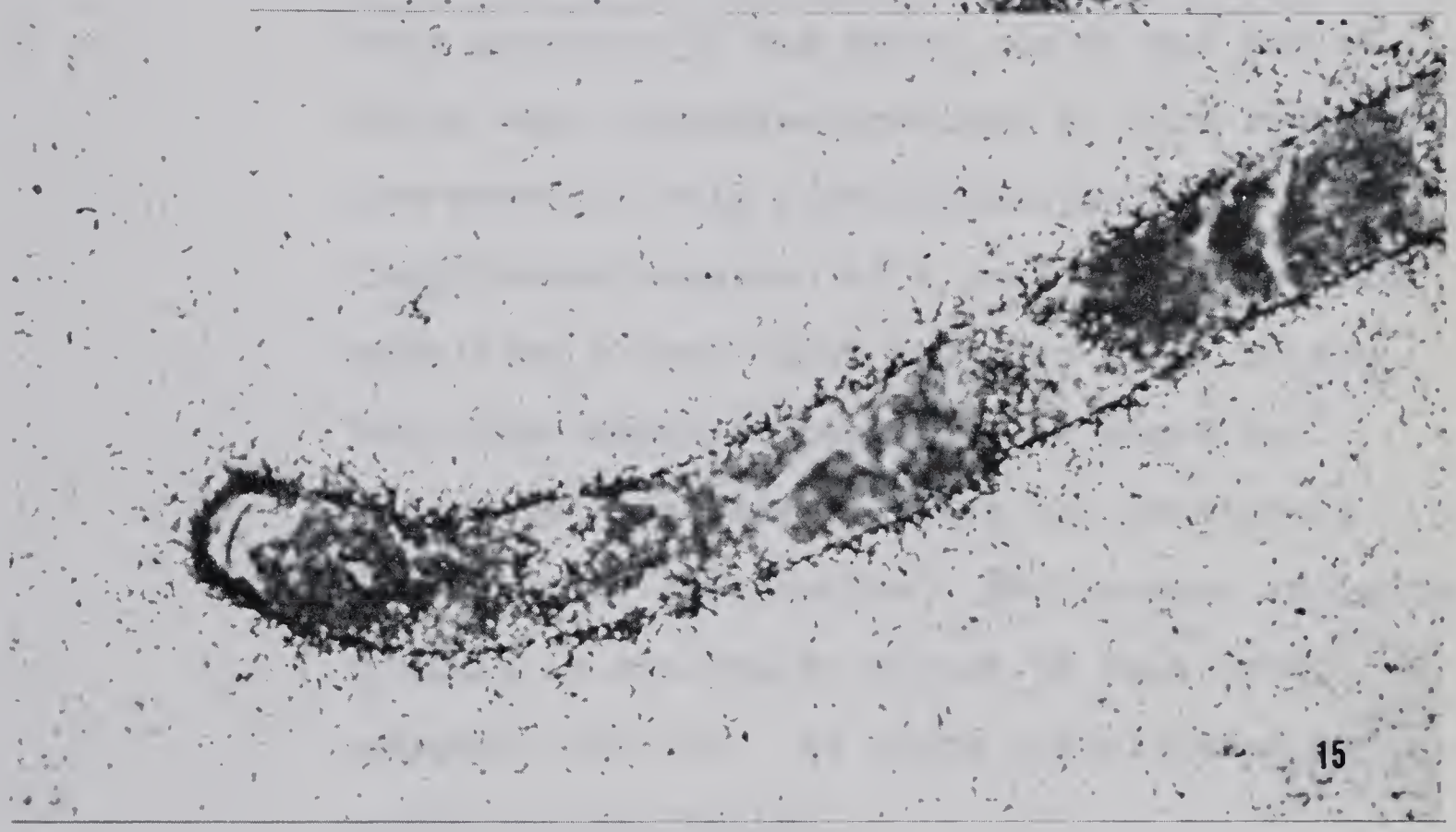
Plate V

FIGURE 14 (X250) Photoradioautograph of a plasmolyzed root hair of a mustard root which was treated by the root-tip immersion method. Radiation is very strong along the whole length of the root-hair wall.

FIGURE 15 (X950) Photoradioautograph of a root hair of a white mustard root grown by the moist-air method. The filter paper was saturated with the $Ca^{45}Cl_2$ solution. Radiation is shown along the wall, in the cytoplasm and in the root-hair dome.

FIGURE 16 (X250) Photoradioautograph of a corn root grown in moist air. The root tip is almost out of focus. Calcification is uniform along the root-hair wall.


14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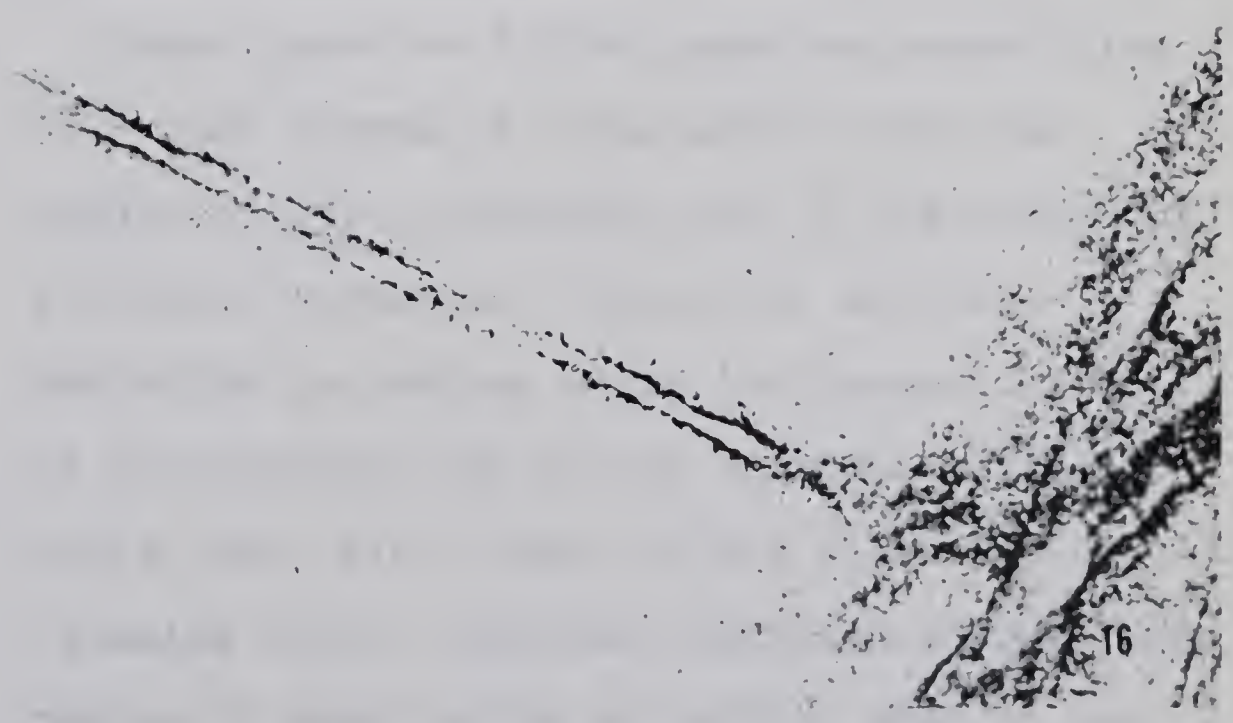
16

solution. The pattern of radioactivity exhibited by a mustard root tip induced by this method is shown in Figure 17. The most intense radiation occurs directly opposite the meristematic region near the root apex. The central white area which represents the approximate position of the stele, is so far out of focus that radiation produced by this region is not shown in this photoradioautograph. A longitudinal section of a young corn root tip made from a root which was also grown by the root-tip immersion technique is shown in Figure 18. In this instance the whole root tip is extremely radioactive. The intense radiation visible in the stelar region of this root suggests that Ca^{45} is being translocated upwards in this region.

Roots grown on filter paper moistened with Ca^{45} show traces of radiation in both the region of cell elongation and in the region of root-hair formation (Figures 19 and 20). Radiation is obvious along the tangential walls of the youngest and as yet hairless epidermal cells (Fig. 19). Then, in the first papilla-forming cells, radiation diminishes until the region of short hairs is reached when it in-

Plate VI

FIGURE 17 (X125) Photoradioautograph of a whole mount of a mustard root tip treated by the root -tip immersion method. Only the emulsion on the sides is in focus. Radiation is most intense opposite the meristematic region.

FIGURE 18 (X125) Photoradioautograph of a longitudinal section of a corn root treated by the root -tip immersion method. Radiation is intense over the tip but soon becomes localized in the stele.

FIGURE 19 (X125) Photoradioautograph of an epidermal strip of a white mustard root treated by the moist-air method. Radiation is present in the cells in the region of elongation, decreases along the papillae-forming cells but increases again in the region of long hairs.

FIGURE 20 (X125) Photoradioautograph of an epidermal strip of a white mustard root treated by the moist-air method. The radiation is very similar to Figure 19. The contrast between the radiation in the region of short hairs and that of the long hairs is more marked.

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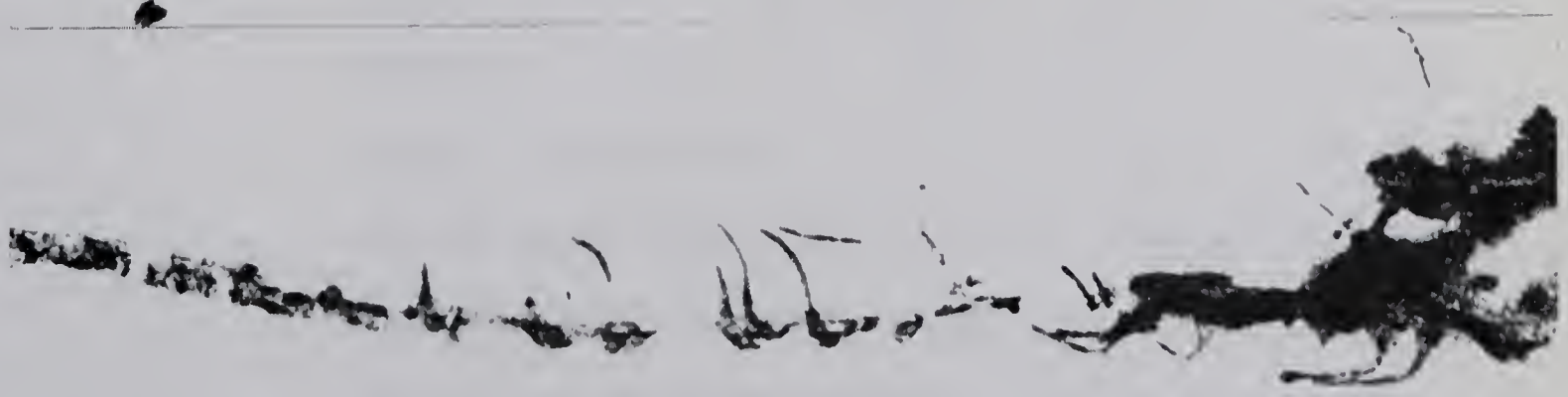
19



20

creases in intensity once more. In general, the papillae and shortest root hairs show less radiation than do the older and longer root hairs. This observation suggests that there is a relationship between the age or the length of a root hair and the amount of radioactivity recorded in the photoradioautograph. Differences in the degree of radiation between the longer hairs and the shorter hairs may be due in part to the fact that the longer hairs are in contact with the moistened surface of the filter paper while the short hairs are elevated above the filter paper by the arching of the root. It has been observed in many experiments that the root tip makes contact with the moistened filter paper and then the root immediately bends or arches upwards only to make contact with the filter paper again in the region of the longer or older hairs. This peculiar tropism is illustrated in Figure 21. Since the root cap is almost impermeable and only a small portion of the root apex is in actual contact with the moistened filter paper, it is believed that only a trace of Ca^{45} enters the root through the root apex and becomes localized in

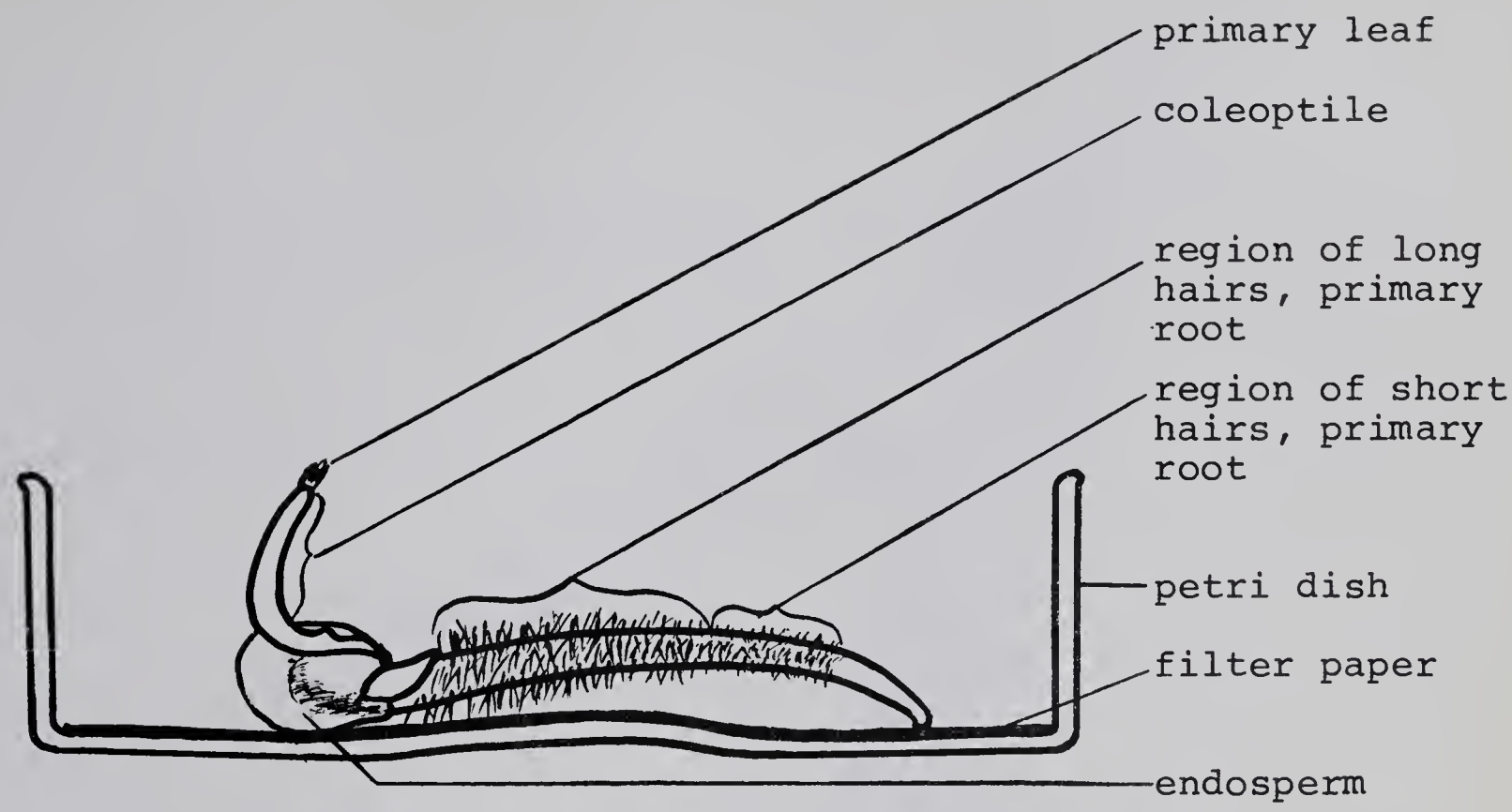
the meristematic region. As mitotic divisions occur, the Ca^{45} concentration in successive root cells diminishes until such a point is reached where the long root hairs are in contact with the moistened filter paper, and, Ca^{45} is absorbed into the root once again. That Ca^{45} is absorbed by the longer hairs is shown by the intense radiation in the region of the longer hairs (Fig. 19). There is an apparent difference in the intensity of radiation between the walls of the hair-forming cells and those that remain hairless (Figs. 22 and 23). On the other hand, the epidermis of the mustard root grown in a strong Ca^{45} solution (Fig. 24) is extremely dark over its whole length. A careful examination of the epidermis of the radioautograph reveals that the long hairless cells are less radioactive than the short hair-forming cells. These differences in radiation are much more evident in transverse sections (Figures 10, 11, 28, and 31).

More information concerning the translocation and distribution of Ca^{45} in the root can be obtained by the study of transverse sections made at different levels from the root apex (Figs. 25 to 31).

Plate VII

FIGURE 21 (X1) Diagram of the moist-air method of treating corn. The tropism displayed at the corn root tip is common in this method of treating seedlings.

FIGURE 22 (X810) Photoradioautographs of a white mustard root hair treated by the moist-air method, where the filter paper is saturated with Ca^{45}. The intense radiation at the root-hair tip shows that Ca^{45} was relatively abundant at this region.


primary leaf
coleoptile
region of long hairs, primary root
region of short hairs, primary root
petri dish
filter paper
endosperm

21

Plate VIII

FIGURE 23 (X810) Photoradioautograph of a strip of mustard epidermis showing a root hair treated by the moist-air method. The long cells on the epidermis are not as strongly radioactive as is the short cell to which the hair is attached.

FIGURE 24 (X810) Photoradioautograph of a strip of epidermis of a white mustard root grown in a $Ca^{45}Cl_2$ solution. Radioactivity is intense and evenly distributed over the walls of the hair-producing cell, the side walls of the hair and over the root-hair dome.

23

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Figure 25 is a photoradioautograph of the section closest to the root apex and shows several rows of root cap cells and a few cells of the central portion of the root meristem. At this level of root development there is essentially no Ca^{45} in either the walls or cytoplasm of the root cap cells but it is present in the walls of the meristematic cells. The greater radioactivity on one side of some of these sections (Figs. 25 and 26) than on the opposite side is due to the fact that they were cut somewhat obliquely. In the next section (Fig. 26) of this series, cut some 140 μ above the root apex, Ca^{45} is localized in the cytoplasm and in the walls of the meristematic cells. The three rows of surrounding root cap cells are scarcely visible. At a higher level still, about 450 μ above the root apex, (Fig. 27) there is greater localization of Ca^{45} in the small cortical intercellular spaces and in the region of xylem differentiation. In the next section (Fig. 28), some 580 μ above the root apex, the intercellular spaces are much larger and appear very dark from radiation. The inner row of intercellular spaces shows greater radiation than the two outer rows. At

Plate IX

FIGURE 25 (X250) Photoradioautograph of a transverse section of a white mustard root 70 μ from the root apex. The section is cut slightly obliquely. Radiation is evident in the region of the meristem nearest the bottom of the picture.

FIGURE 26 (X250) Photoradioautograph of a transverse section of the same white mustard root as that of Figure 25, 140 μ from the root apex. Radiation is quite intense above the meristematic cells.

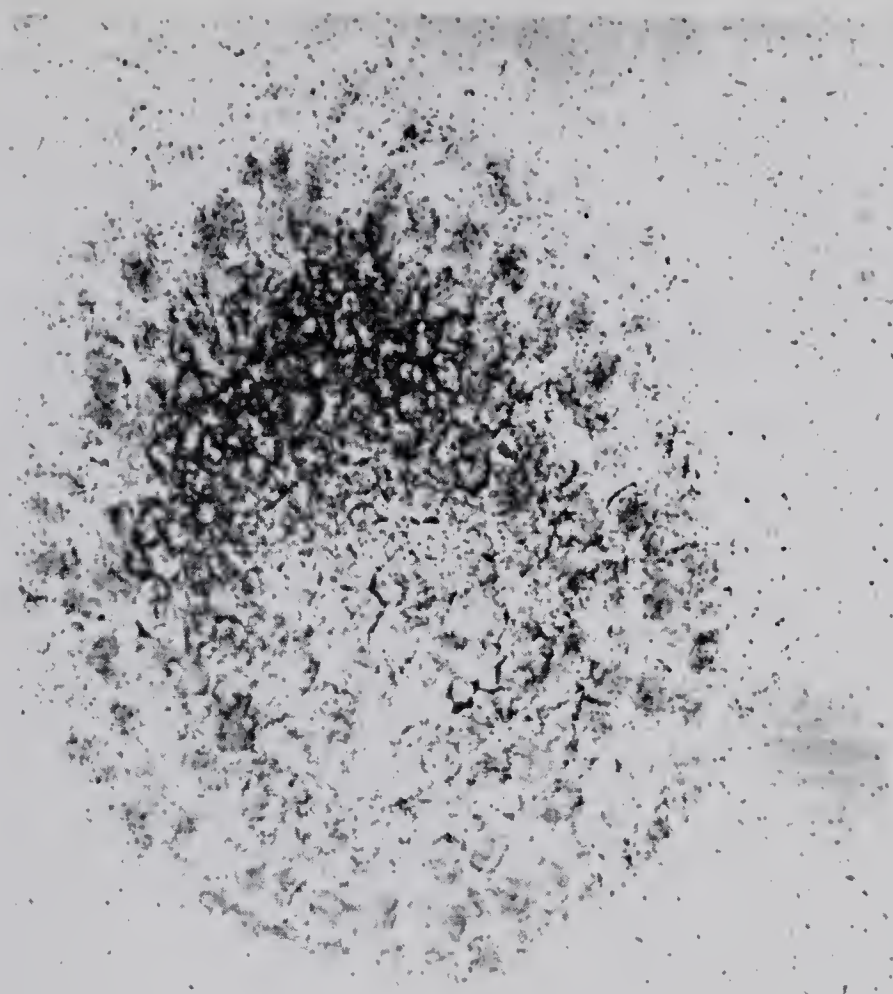

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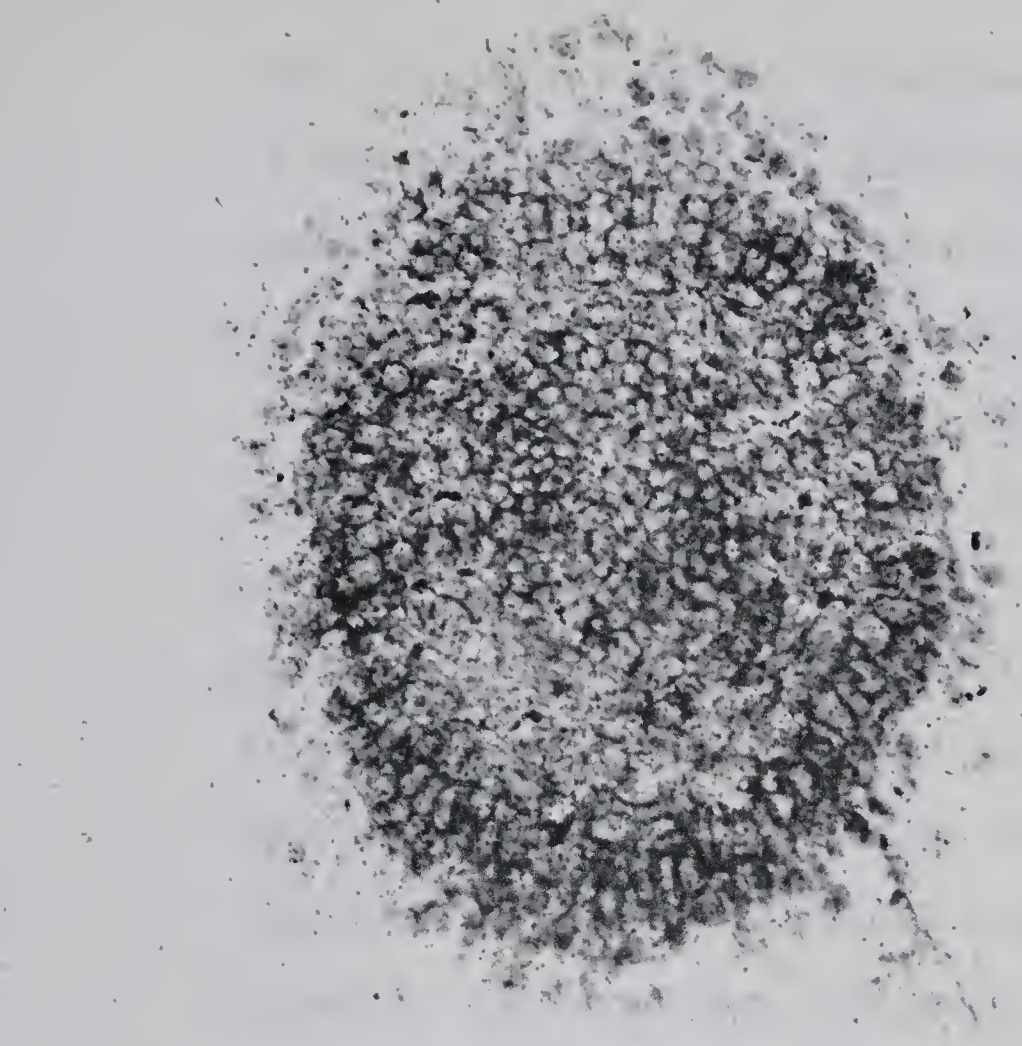
Plate X

FIGURE 27 (X250) Photoradioautograph of a transverse section of a white mustard root, treated by the moist-air method, cut 450 μ from the root apex. Small cortical intercellular spaces show conspicuous darkening. The slightly more intense radiation in the stele is above the xylem.

FIGURE 28 (X250) Photoradioautograph of a transverse section of a white mustard root treated by the moist-air method, cut 580 μ from the root apex. A short cell is indicated by the arrow. The radiation above the intercellular spaces is much more intense than in Figure 27.



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28

this level of root-tip development, two rows of root-cap cells still surround the root proper and the epidermis is differentiated already into long and short cells.

In the transverse section shown in Figure 29, which was cut some 900 μ from the root apex, radiation in the extremely small intercellular spaces is obvious, but not dense. It would appear from an examination of this photoradioautograph (Fig. 29) that Ca^{45} has definitely reached the short cells lying directly opposite the intercellular spaces. In the next transverse section from the same root, cut at a distance of about 1300 μ above the root apex, (Figure 30), Ca^{45} is much more strongly concentrated in the intercellular spaces nearest the stele. There is also a well defined border of radiation surrounding the transverse section. But since this root was grown directly in contact with Ca^{45} this outer border of radiation may not signify that Ca^{45} is bound to the pectic materials of the epidermal cell walls as $calcium^{45}$ pectate.

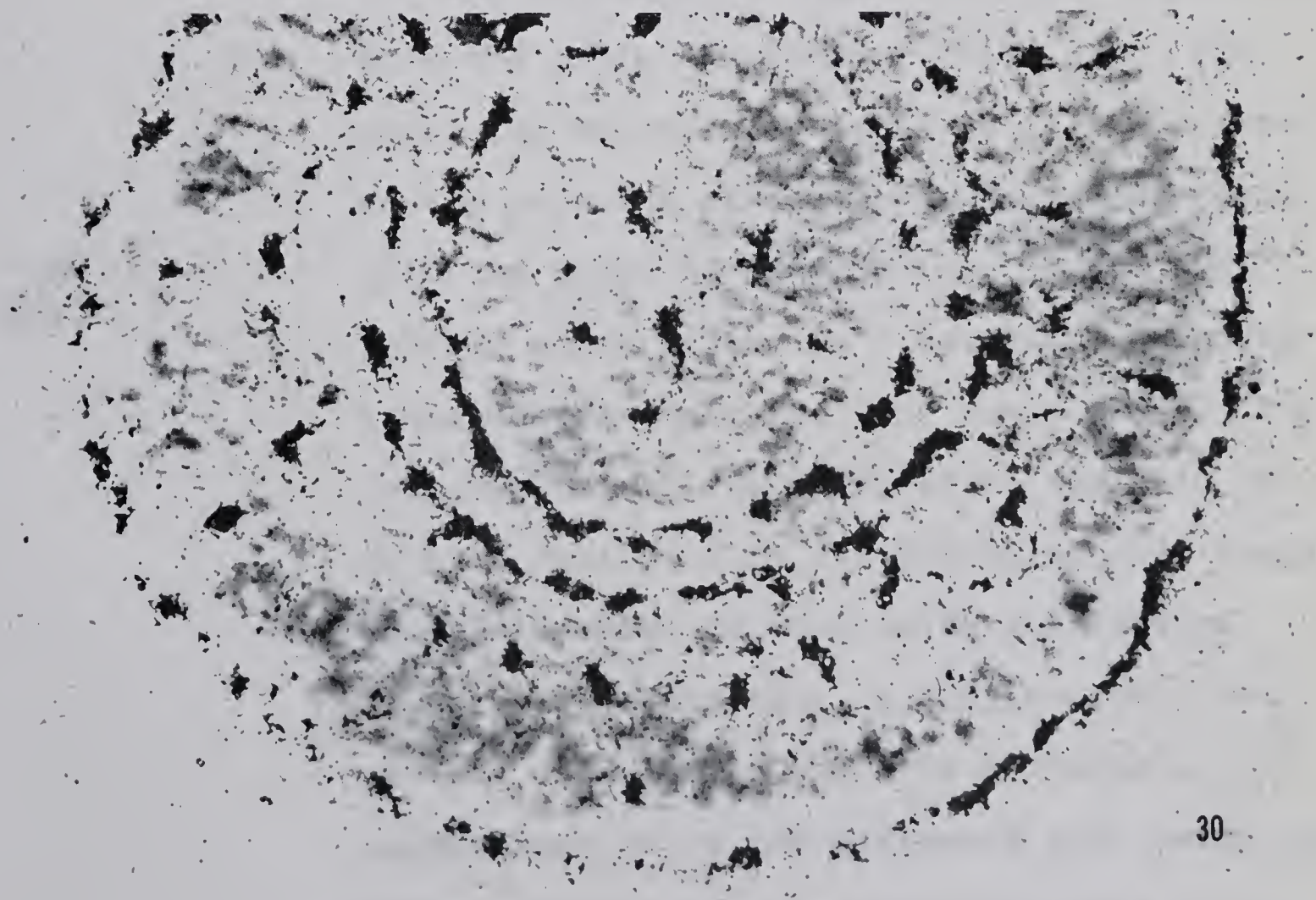
The transverse section (Figure 31) cut from a different root than Figures 29 and 30 but

Plate XI

FIGURE 29 (X250) Photoradioautograph of a transverse section of a white mustard root 900 μ from the root apex. Radiation from the inner row of intercellular spaces and the walls between these spaces, almost form a complete ring of radiation. Radiation extends back from this inner ring of radiation in radial lines along the radial cell walls to the short cells.

FIGURE 30 (X250) Photoradioautograph of a transverse section of a white mustard root (from the same root as Figure 29) 1300 μ from the root apex. There are three rows of radiation from the intercellular spaces. Surrounding the root is a row of radiation believed to be adsorbed Ca^{45}.

29



30

treated in the same way, is from the region of mature root hairs. Radiation is largely confined to the stele and the epidermis, with faint rays of radiation extending from the short cells in the epidermis to the stele. Areas of intense radiation in the epidermis represent the position of the short cells.

The translocation of Ca^{45} from the seed to the root tip may be compared with the translocation of leucine. Earlier work with leucine has shown that this substance is translocated downward into the root extending to the apex (34). Ca^{45} is different in that it is not translocated down the stele of the root (Fig. 32). The radiation visible in Figure 32 is due entirely to the movement of Ca^{45} by surface adsorption from the point of introduction. When boric acid is added to the Ca^{45} solution, downward translocation into the root takes place. This observation will be referred to again in the section dealing with the Geiger counter studies.

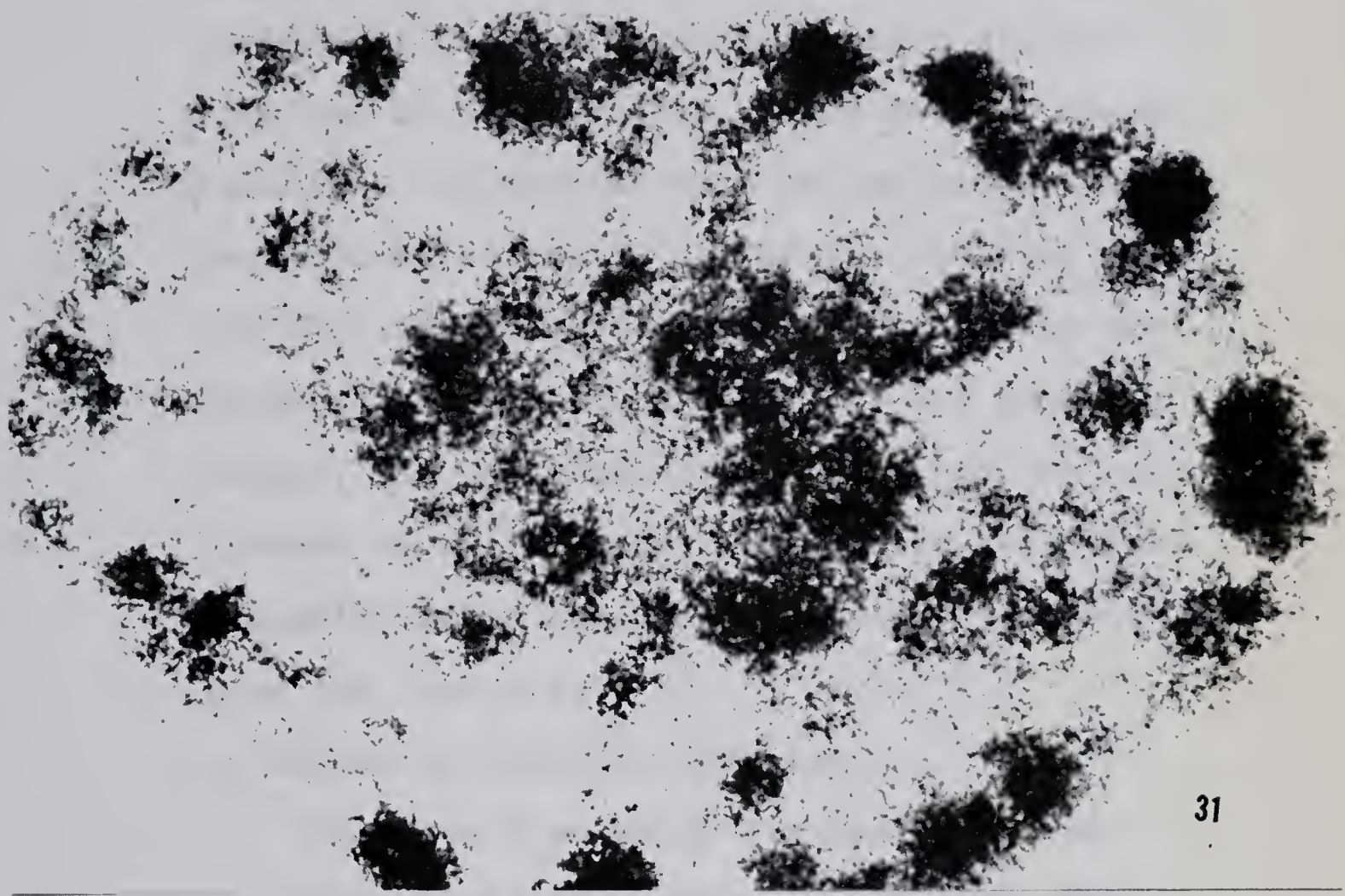
B. Non-Radioactive Calcium Culture Solutions

For each experiment, a longitudinal root section is selected which is believed to be representative of the different root sections. These sections will be described best with

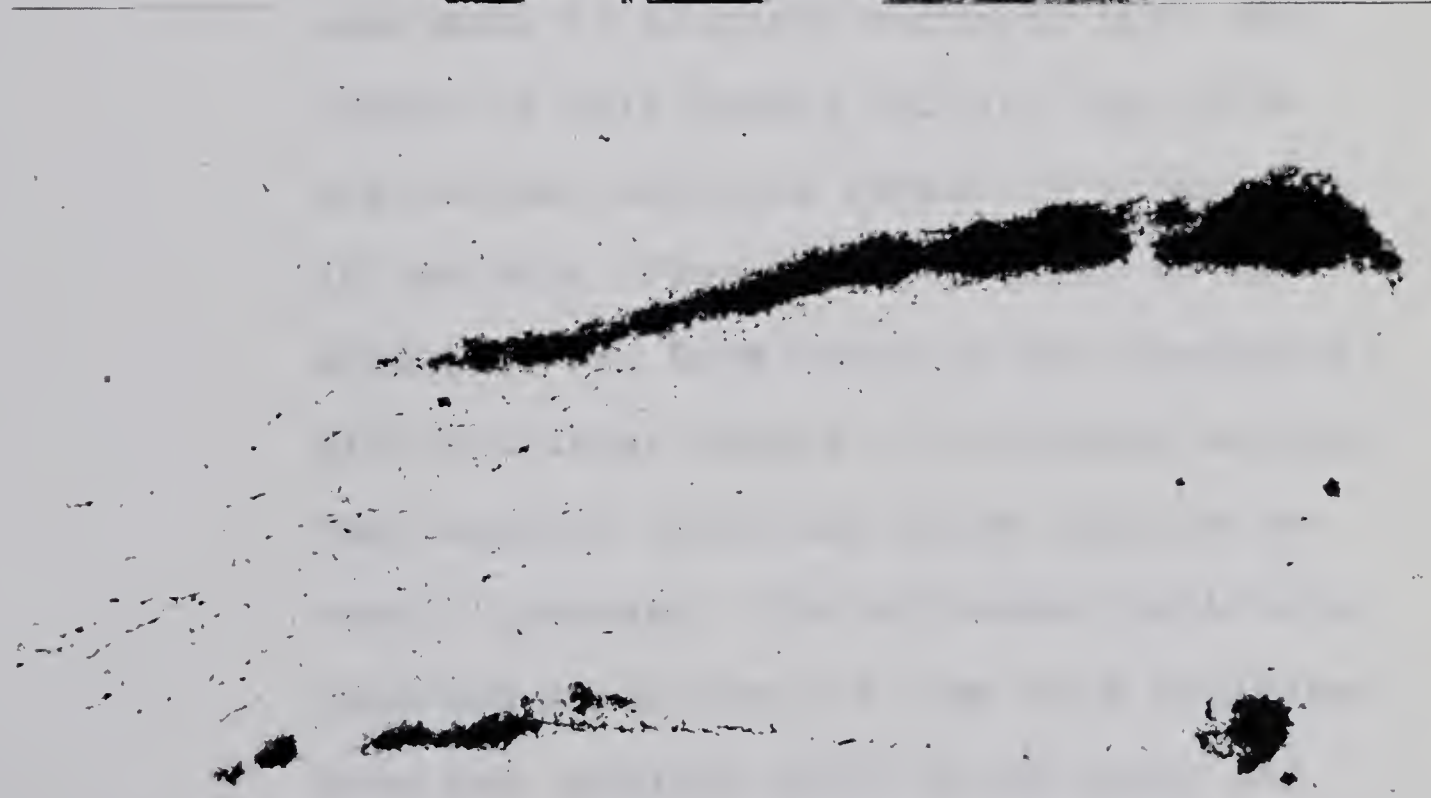
Plate XII

FIGURE 31 (X250) Photoradioautograph of a transverse section of a white mustard root 1900 μ from the root apex, (region of mature root hairs). Radiation is intense above the short cells of the epidermis and in the stele. Radial rays of radiation extending from the short cells to the stele are also obvious.

FIGURE 32 (X80) Photoradioautograph of a longitudinal section of a corn root immediately next to the seed. The root was treated by introducing calcium through a hole bored in the endosperm. The radiation along the root epidermis is from adsorbed calcium^{45}.



31



32

reference to photomicrographs. Since only a portion of the longitudinal section of the root can be photographed at the desired magnification, the section will be represented by two photomicrographs, one of the root tip and the other of the mature region of the root. Since roots in culture solutions are commonly curved, the longitudinal sections are not continuous so it is impossible to have the second photomicrograph begin at a specific distance from the root tip.

1. Series of Disodium versenate

The root from which Figures 39 and 40 were made is slightly narrower than the others of this series and all the cells are smaller and more compactly arranged. In the root (Figs. 33, 34) grown in the strongest Na_2 EDTA solution and therefore with the least amount of available calcium, the cortical cells are large and are unevenly arranged. The epidermal cells are compressed and some of them have separated from the cortical cells along their end walls. Root hairs are sparsely developed. When present they are not much longer than the papillae and are thin walled. In the

root (Figs. 35, 36) grown in a less concentrated Na_2 EDTA solution, the cortical cells (Fig. 36) are more evenly arranged than in Figure 34, but as characteristic of roots grown in solutions deficient in calcium, their end walls are noticeably undulated. The epidermal cells of Figures 35 and 36 are also more normal in appearance although the wall is definitely bulged at the base of a papilla. Root hairs are more numerous, longer and straighter than in roots grown in stronger Na_2 EDTA solution. The next root (Fig. 37, 38), grown in a less concentrated Na_2 EDTA solution, shows no sign of calcium deficiency. The epidermal and cortical cells are evenly arranged with regular and straight walls. The overall width of this root is conspicuously greater than that of either of the other two roots (Figs. 33, 34 and 35, 36). In the root (Figs. 39, 40) grown in the least concentrated Na_2 EDTA solution, the cortical cells are smaller and arranged in very straight rows. The epidermal cells are relatively large, hairless and evenly arranged in long rows.

2. Series of pH

Early vacuolation, irregular undulated cell

Plate XIII

All of the photomicrographs are longitudinal sections of white mustard roots grown in saturated solutions of $CaSO_4$ buffered at pH 8.2, with 1 cc. of boric acid added to each solution and varying amounts of Na_2 EDTA. Each pair of photomicrographs at one level on the plate are of the same root grown in a particular concentration of Na_2 EDTA.

FIGURES 33 and 34 — Photomicrographs of roots grown in the culture solution with 30 cc. of saturated Na_2 EDTA solution added. Figure 33 is a photomicrograph of the root tip. Figure 34 is a photomicrograph of a section of the same root as Figure 33 beginning at 3.18 mm's from the root apex. The epidermis is compressed. Cortical cells are large and the walls are very irregular, often being difficult to trace.

FIGURES 35 and 36 — Photomicrographs of roots grown in the culture solution with 10 cc. of saturated Na_2 EDTA solution added. Figure 35 is a photomicrograph of the root tip.

Plate XIII

(continued)

Figure 36 is a photomicrograph of a section of the same root as Figure 35, beginning at 8.41 mm's from the root apex. The cortical cells are more evenly arranged than those in Figure 34, although the end walls are undulated.

FIGURES 37 and 38 — Photomicrographs of a root grown in the culture solution with 2.5 cc. of saturated Na_2 EDTA solution added. Figure 37 is a photomicrograph of the root tip. Figure 38 is a photomicrograph of a section of the same root as Figure 37, beginning at 5.21 mm's from the root apex. The cells are in even rows with little undulation of the end walls.

FIGURES 39 and 40 — Photomicrographs of roots grown in the culture solution with 1.0 cc. of saturated Na_2 EDTA solution added. Figure 39 is a photomicrograph of the

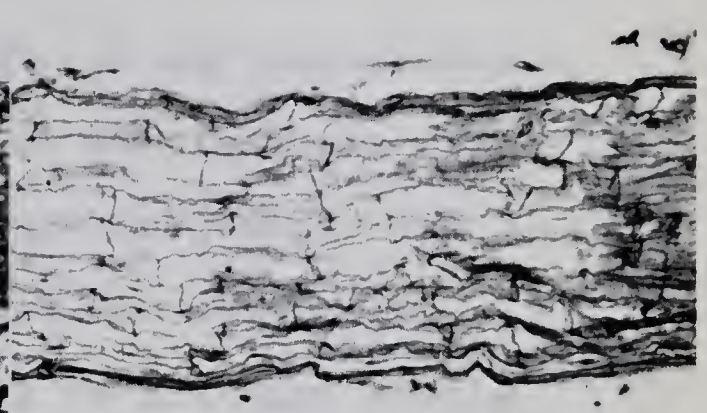
Plate XIII

(continued)

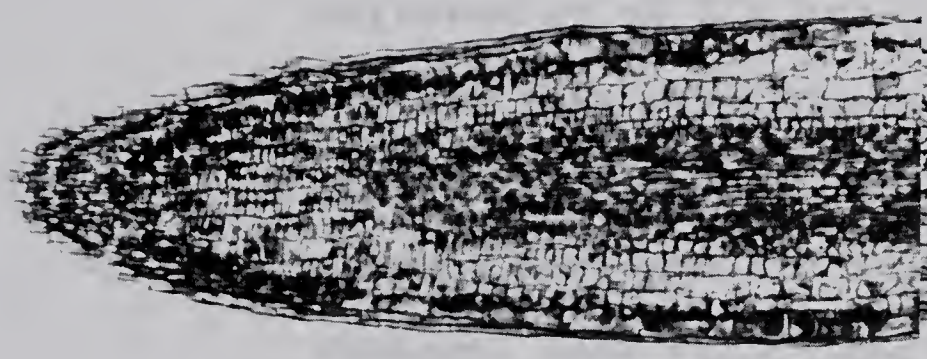
root tip. Figure 40 is a photomicrograph of a section of the same root, beginning at 3.55 mm. from the root apex. The epidermal cells are large, and evenly arranged in long rows. The root is much narrower than any of the other roots.

The magnification of Photomicrographs 33 - 40 is X95.

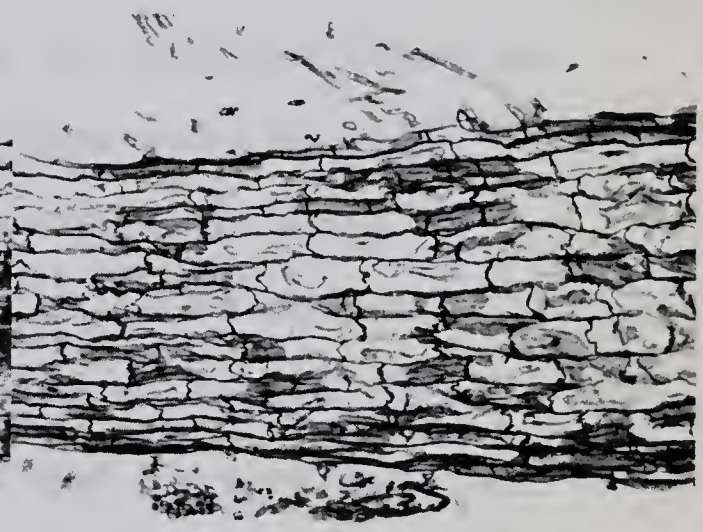
33



34



35



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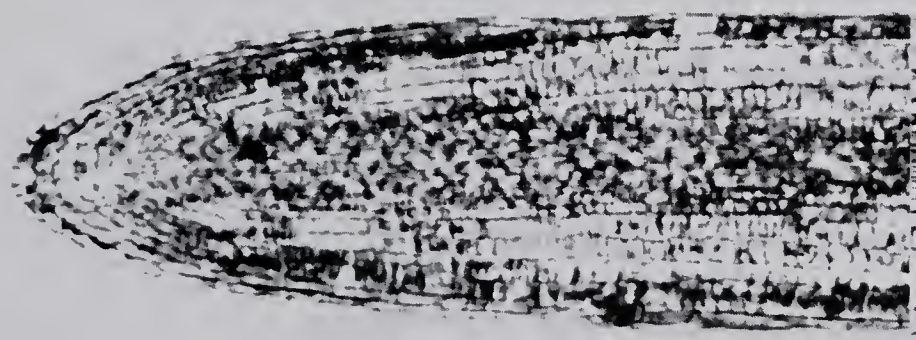

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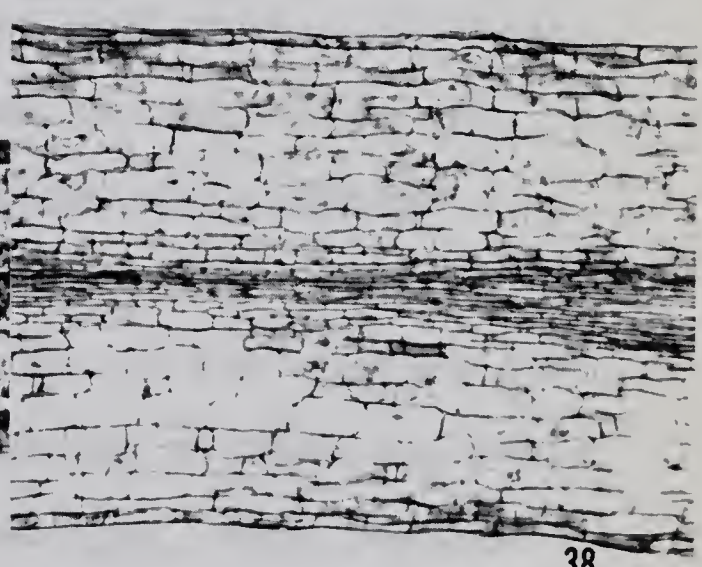

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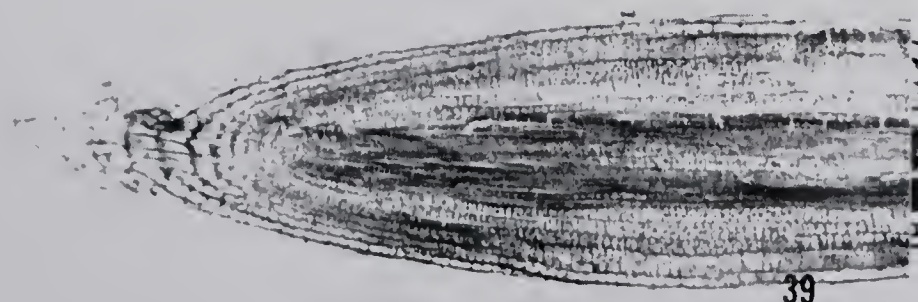


39

40

walls, and a typical root cap and meristematic region (Figures 41 and 42) are features of a pH of 7.6 with this culture solution. The epidermal cells are bulged on their outer tangential wall, the ends do not meet evenly and there are few papillae. The sections (Figures 43 and 44) from the culture solution, buffered at pH 9.4 are comparable to well -formed root sections and show no signs of calcium deficiency. The cells of the root cap and meristematic region of Figure 43 are compact, less vacuolated and have straighter walls than those of Figure 41. The straight epidermal cell walls with well-formed papillae and brick-like appearance of the cortex (Fig. 44) are signs of sufficient calcification.

3. Series of Boric Acid

The greater calcification of the mustard root grown in the culture solution without any boric acid is evident by the smaller, more regular cells with very straight cell walls (Fig. 46). The roots grown in a culture solution with 0.2×10^{-16}M boric acid (Fig. 48) on the contrary, has irregular cells with thin undulated walls. The epidermis is very poorly outlined.

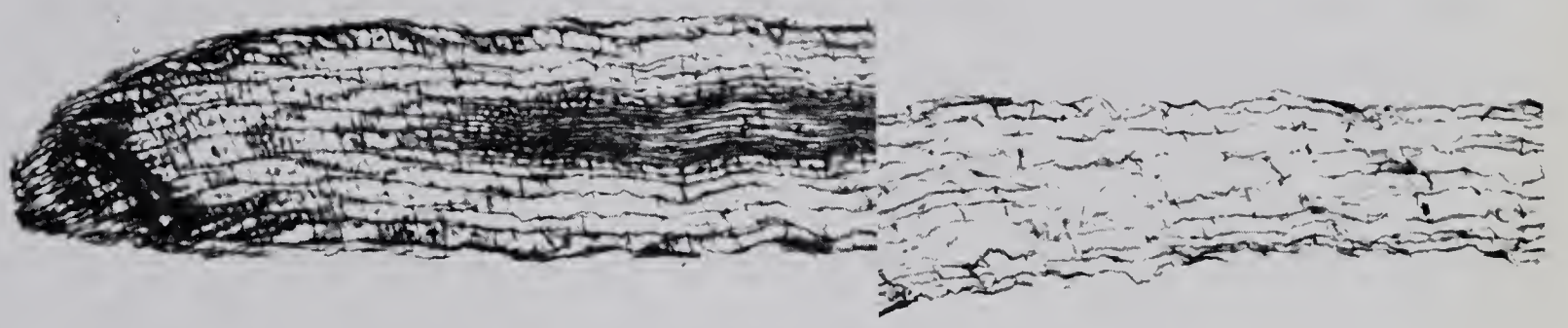
The cracked epidermis and the small uniformly arranged cells of the cortex of the corn root

Plate XIV

All of the photomicrographs are longitudinal sections of white mustard roots cultured in saturated $CaSO_4$ solutions with different concentrations of tris buffer (pH). Each pair of photomicrographs at the one level on the plate are from the same root. (X95).

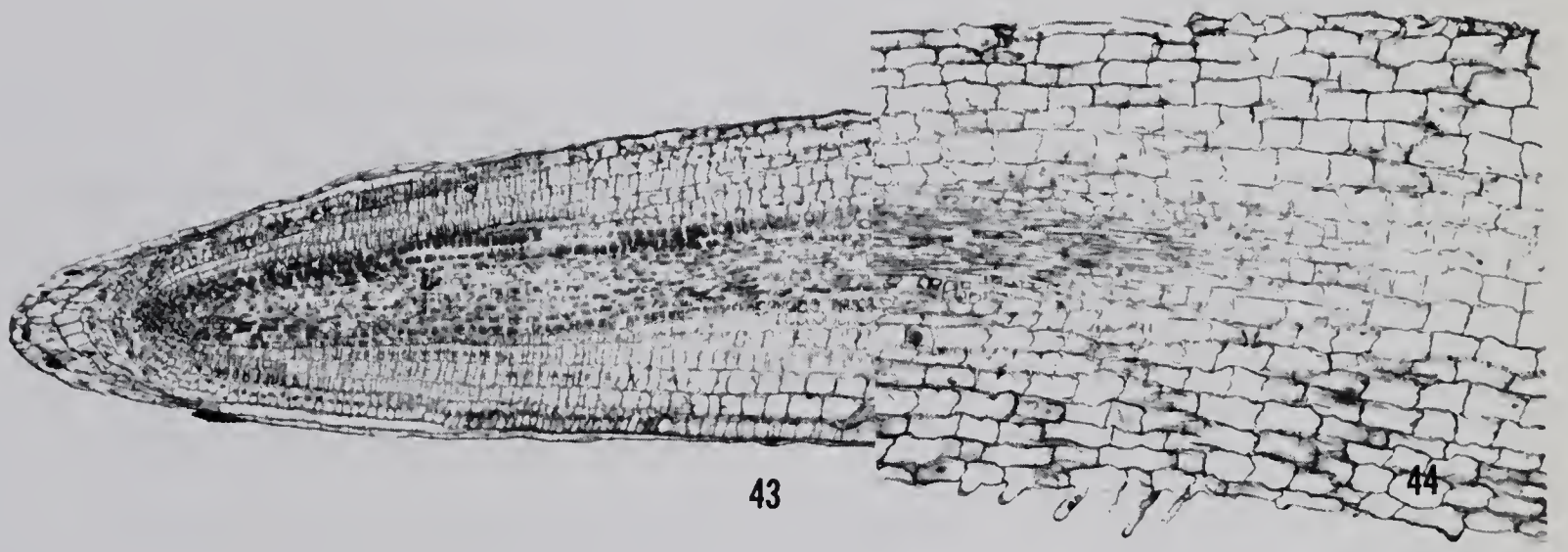
FIGURES 41 and 42 — Photomicrographs of a longitudinal section of a white mustard root grown in a culture solution with a tris buffer of pH 7.6. Figure 41 is of the root tip. Figure 42 is a segment of the non-calcified root, beginning at 2.05 mm's from the root apex.

FIGURES 43 and 44 — Photomicrographs of a longitudinal section of a white mustard root grown in a culture solution with a tris buffer of pH 9.4. Figure 43 is a photomicrograph of the root tip. Figure 44 is a photomicrograph of a calcified root, beginning at 2.14 mm's from the root apex. The cells are brick-like and papillae are formed.



41

42



43

44

Plate XV

Photomicrographs (Figures 45 - 48) are longitudinal sections of white mustard grown in 10^{-2} $CaSO_4$ solutions with different concentrations of boric acid.

FIGURES 45 and 46 — Photomicrographs of roots grown in a culture solution with no boric acid. Figure 45 is a photomicrograph of the root tip. Figure 46 is a photomicrograph of a root, beginning at 4.97 mm's from the root apex, showing characteristics of calcified walls.

FIGURES 47 and 48 — Photomicrographs of roots grown in a culture solution with 0.2 x 10^{-16}M boric acid added. Figure 47 is a photomicrograph of the root tip. Figure 48 is a photomicrograph of a root, beginning at 4.62 mm's from the root apex showing signs of calcium deficiency.

FIGURES 49 and 50 — Photomicrographs of a corn root grown in a culture solution with no boric acid. Figure 49 is a photomicrograph of a root tip. Figure 50 is a photomicro-

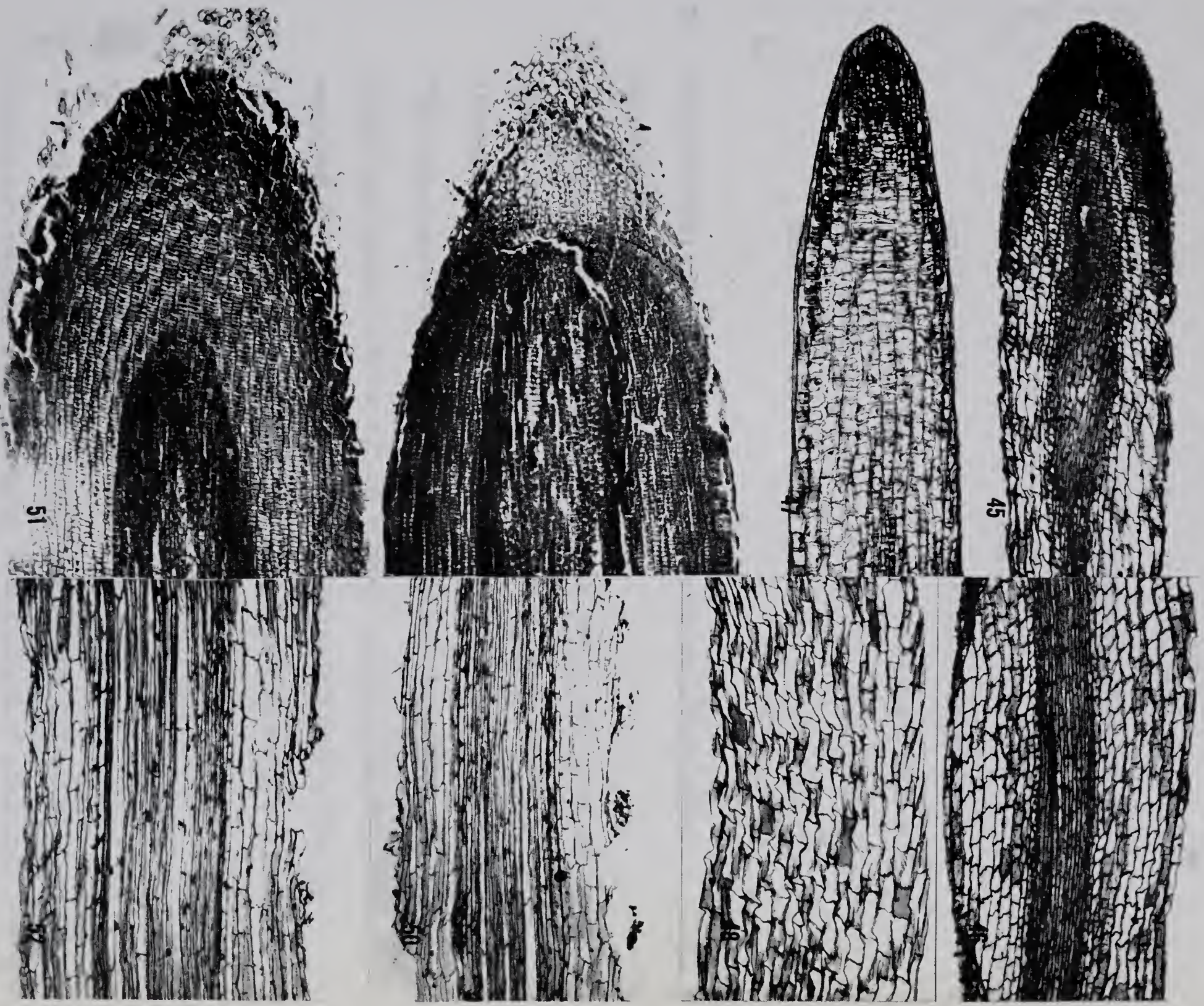
Plate XV

(continued)

graph of a root, beginning at 6.8 mm. from the root apex showing strong calcification of the walls.

FIGURES 51 and 52 — Photomicrographs of a corn root grown in a culture solution with 0.2 x 10^{-16}M boric acid added. Figure 51 is a photomicrograph of the root tip. Figure 52 is a photomicrograph of a root, beginning at 5.25 mm. from the root apex showing only slight calcification of the walls.

The magnification of Photomicrographs 45 - 52 is X95.


51
57
53
56
45
47

(Fig. 50) grown in a culture solution without boric acid is also characteristic of over-calcification. The corn root grown in 0.2 x 10^{-16}M boric acid (Fig. 52) with its larger cells, less prominent walls, and more undulated end walls of the cortical cells is characteristic of a slightly less calcified root.

III. Translocation and Distribution Studies Using the Geiger Mueller Counter

Figure 53 records the results obtained from assaying a corn root which was treated by the root-tip immersion method and cut into 3 millimeter lengths. The radioactivity increases progressively from the root-cap region where it is almost negligible to section 8 (24 mm. from the root apex) of the primary root. The remaining 9 mm. of the corn root has a diminishing amount of radiation. The reduction in radiation continues on from the root through the transition zone to the shoot apex.

Five mustard roots, also treated by the root-tip immersion method and cut into 1 millimeter lengths, show similar results to those of the corn roots (Figure 54). Radiation progressively increases from the root cap to 11 mm. from the root apex which is almost at the end of the root hair region. The hypocotyl has pro-

FIGURE 53 Radiation of Ca^{45} in counts per minute per milligram of 3 millimeter serial sections from the root apex through to the shoot apex of a corn seedling. The root was treated by the root-tip immersion method. Sections 1 to 11 are of the primary root. Sections 12 and 13 are of the transition region. Sections 14 to 17 are of the shoot.

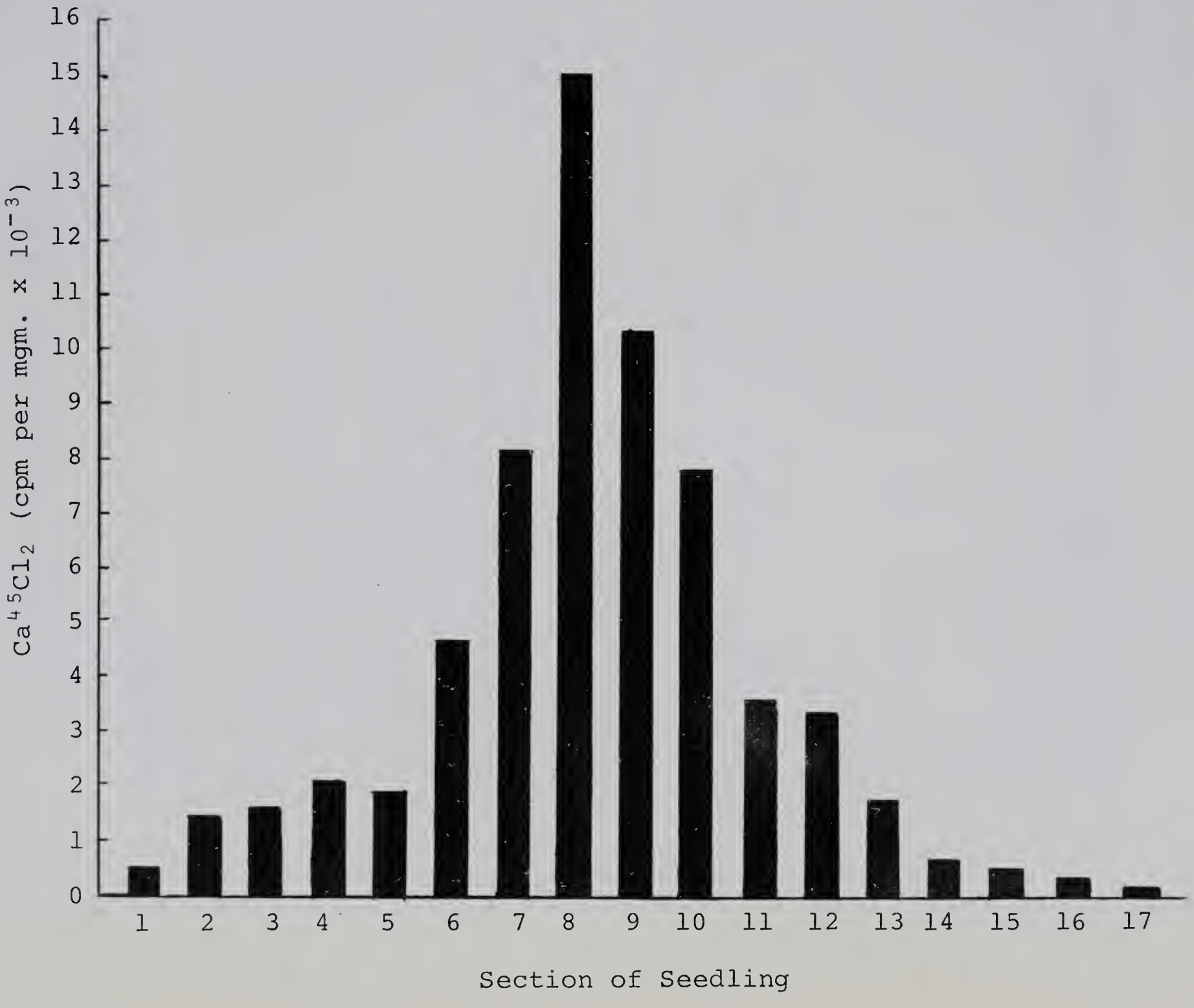
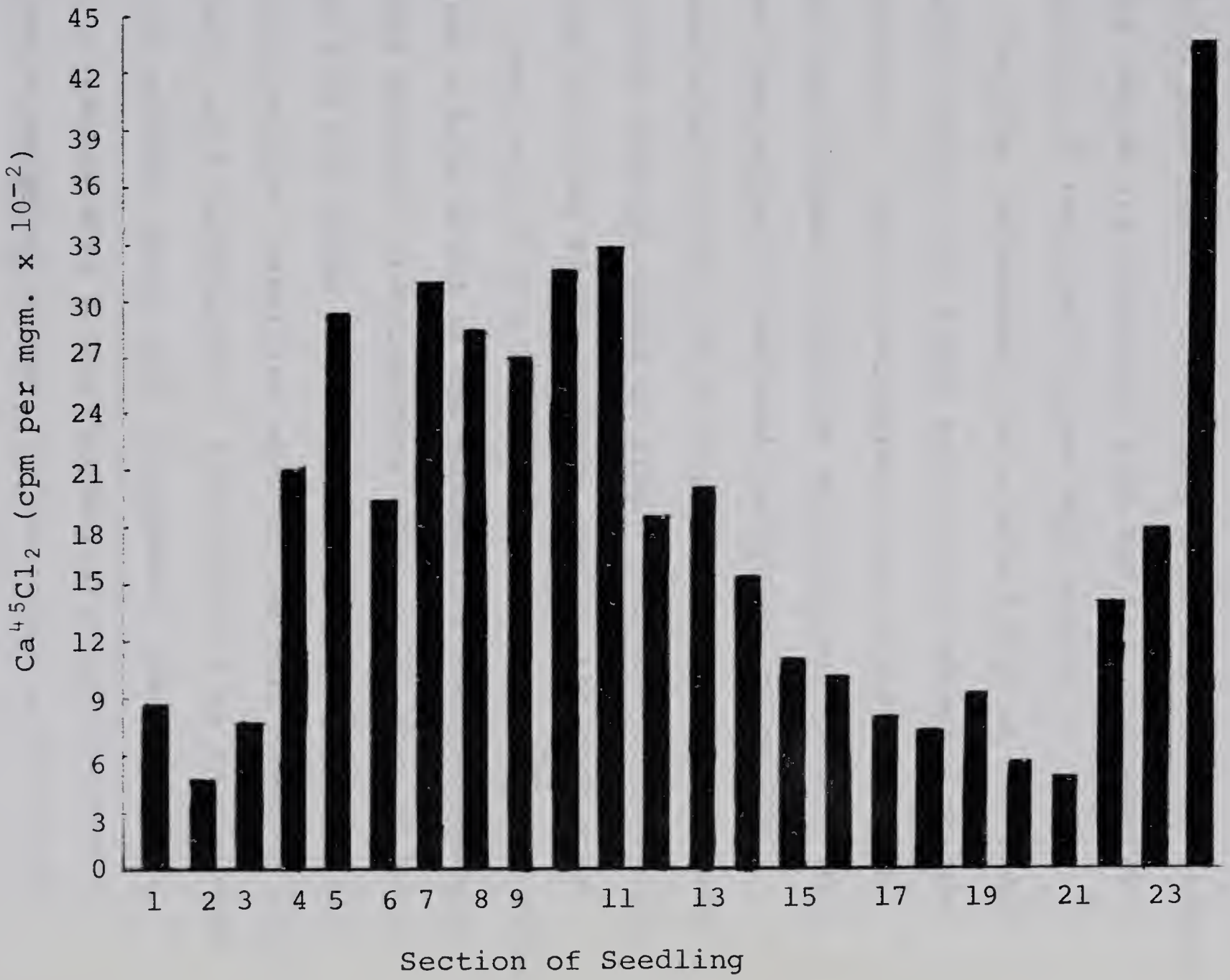

16
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
$Ca^{45}Cl_2$ (cpm per mgm. x 10^{-3})
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17
Section of Seedling

FIGURE 54 Radiation of Ca^{45} in counts per minute per milligram of 1 millimeter serial sections of 5 seedlings of white mustard. Seedlings were sectioned from the root apex through to the first foliage leaves. The root was treated with $Ca^{45}Cl_2$ by the root-tip immersion method. Sections 1 to 15 are of the root. The root-hair region terminated at 13 millimeters. Sections 16 to 23 are of the hypocotyl. Section 24 is of the leaves.


45
42
39
36
33
30
27
24
21
18
15
12
9
6
3
0
Ca45Cl2 (cpm per mgm. x 10-2)
1 2 3 4 5 6 7 8 9 11 13 15 17 19 21 23
Section of Seedling

gressively diminishing amounts of radiation up to 21 mm. from the root apex. The last 2 mm. of the hypocotyl have rapidly increasing amounts of radioactivity. The leaves are observed to be the most radioactive portion of the seedling per unit weight.

Calcium introduced into a hole bored in the endosperm (Figure 55) shows very little downward translocation. The two sections of the transition zone and one section in each of the adjacent primary root and the epicotyl have weak radiation (below 2100 counts per minute per milligram). There is, however, a very constant trend of slight increase of radiation from the base of the primary root to the root apex.

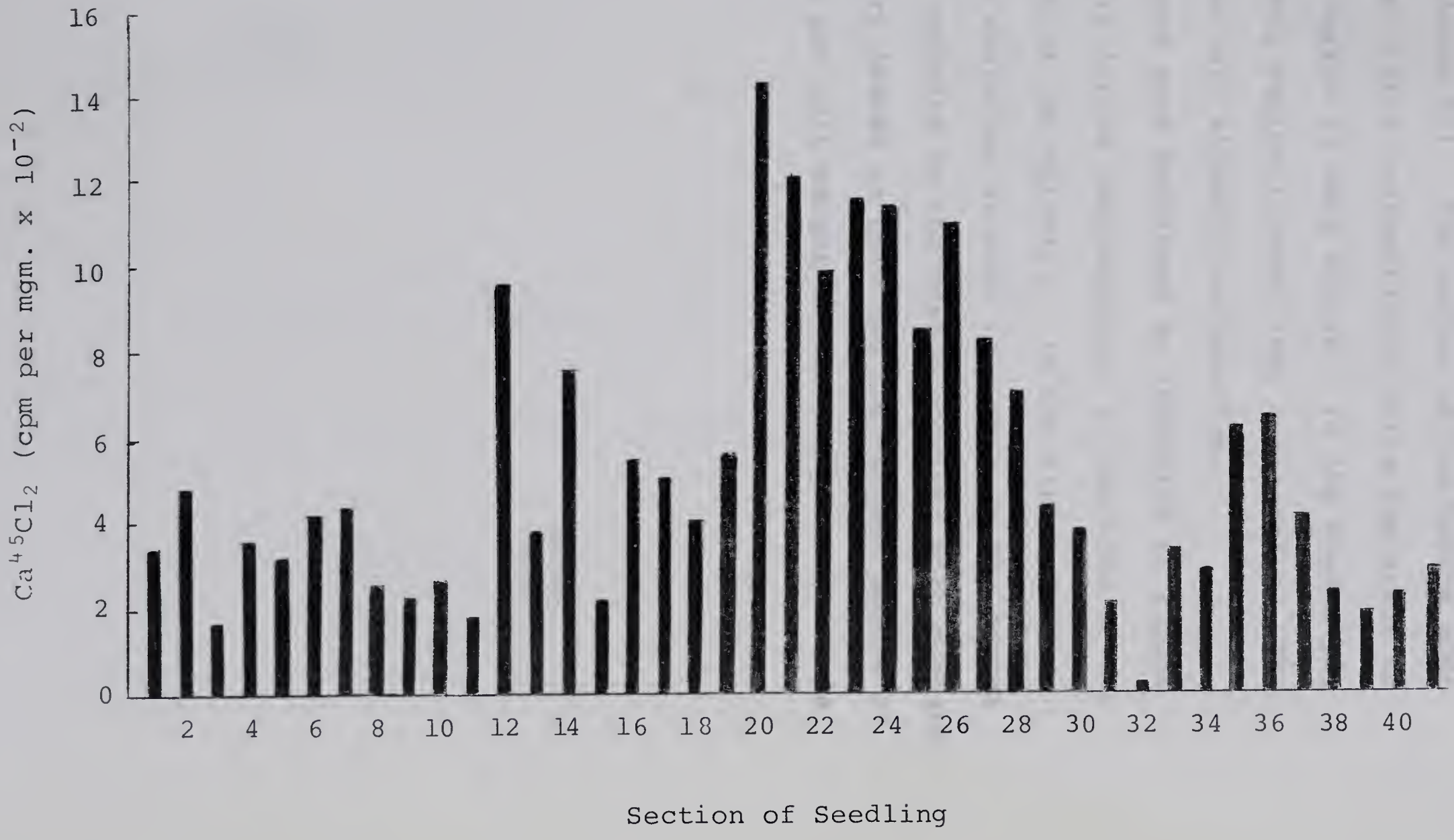
Boric acid (0.2×10^{-2}M) added to the Ca^{45} introduced into a hole bored in the endosperm allows considerably more downward translocation (Figure 56) than if boric acid is not added to the Ca^{45} (Figure 55). The radiation at the transition zone of the corn seedling with boric acid introduced is lower than it is in the seedling without boric acid introduced, however the Ca^{45} has translocated a considerably greater distance on each side of the transition zone than in the former experiment.

Mustard roots labelled with Ca^{45} by the root-tip immersion method, sectioned into regions, and where possible the stele separated from the cortex, showed that the meristematic region is only slightly radio-

FIGURE 55 Radiation of Ca^{45} in counts per minute per milligram of 3 millimeter serial sections from the root apex through to the shoot apex of a corn seedling. The root was treated by introducing $Ca^{45}Cl_2$ into a hole bored into the endosperm of the seed. The seedling was grown on moist filter paper. Sections 1 to 23 are of the root. Sections 24 and 25 are of the transition zone between the root and shoot. Sections 26 to 30 are of the shoot.


$Ca^{45}Cl_2$ (cpm per mgm. x 10^{-2})
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
2
4
6
8
10
12
14
16
18
20
22
24
26
28
30
Section of Seedling

FIGURE 56 Radiation of Ca^{45} in counts per minute per milligram of 3 millimeter serial sections from the root apex through to the shoot apex of a corn seedling. The root was treated by introducing $Ca^{45}Cl_2$ and 0.2×10^{-2}M boric acid into a hole bored into the endosperm of the seed. Sections 1 to 20 are of the primary root. Sections 21 to 42 are of the shoot.


$Ca^{45}Cl_2$ (cpm per mgm. x 10^{-2})
16
14
12
10
8
6
4
2
0
2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38 40
Section of Seedling

active (Figure 57). The cortex of the mature root region has little radioactivity while the stele of the same region is very active. In the hypocotyl, the cortical region is much less radioactive. The leaves are only slightly radioactive.

The corn root sectioned as indicated by Figure 58 had very little radioactivity in the root or the coleoptile of the epicotyl. In the stele of this root, the radiation is even less than in the cortex which is opposite to the result obtained with mustard. The primary leaves in corn had the largest amount of radiation per unit weight of all the pieces in this seedling.

FIGURE 57 Radiation of Ca^{45} in counts per minute per milligram of five mustard seedling pieces as indicated below. The root was treated with $Ca^{45}Cl_2$ by the root-tip immersion method.

Piece 1: Root cap, meristematic and elongation region. Average length of piece is 1.7 mm.

Piece 2: Cortex of the hair region. Average length of piece is 12.2 mm.

Piece 3: Stele of the hair region. Average length is 12.2 mm.

Piece 4: Cortex of the hypocotyl. Average length is 10.4 mm.

Piece 5: Stele of the hypocotyl. Average length is 10.4 mm.

Piece 6: Primary foliage leaves.

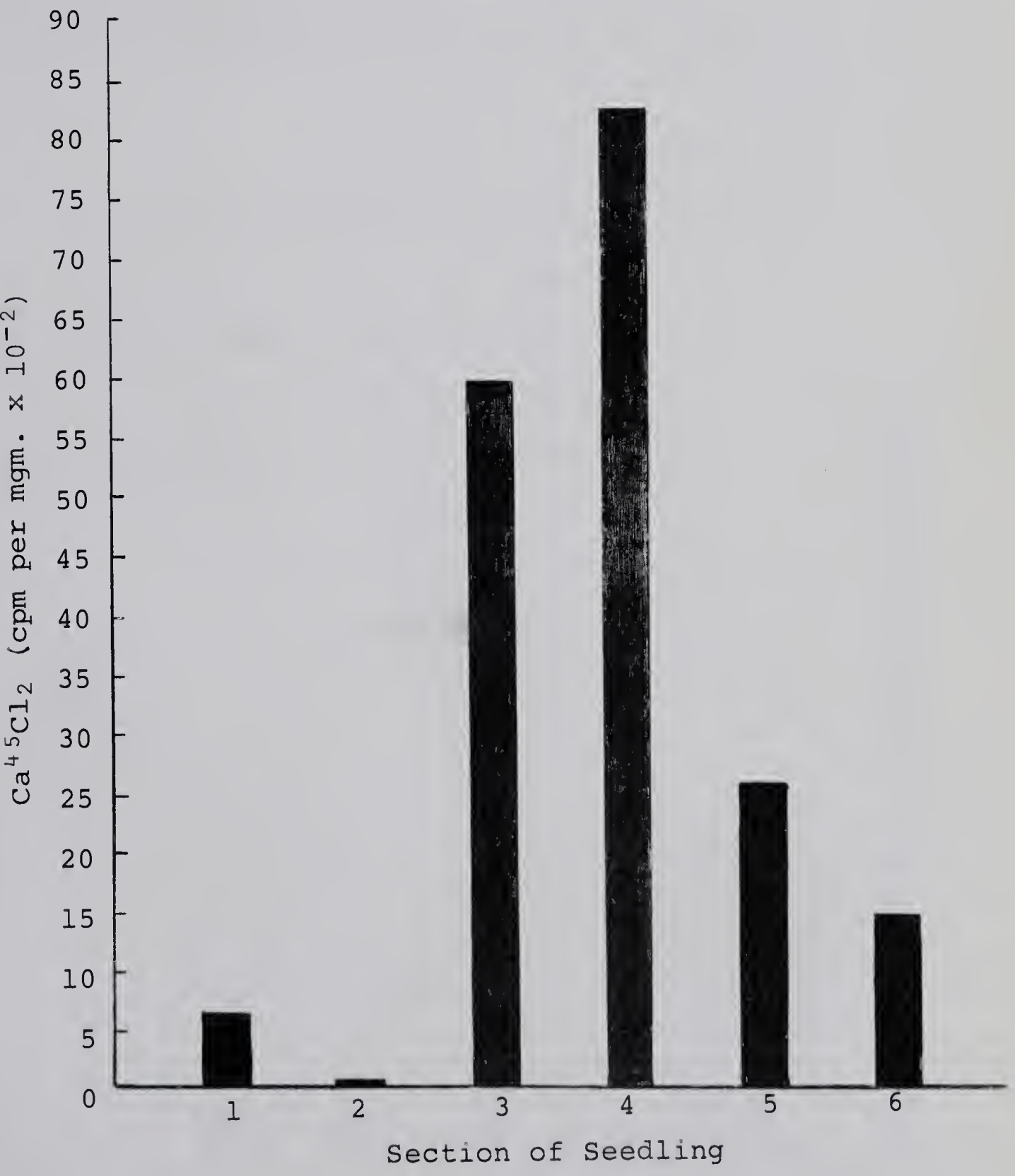

90
85
80
75
70
65
60
55
50
45
40
35
30
25
20
15
10
5
0
$Ca^{45}Cl_2$ (cpm per mgm. x 10^{-2})
1
2
3
4
5
6
Section of Seedling

FIGURE 58 Radiation of Ca^{45} in counts per minute per milligram of corn seedling pieces as indicated below. The root was treated with $Ca^{45}Cl_2$ by the root-tip immersion method.

Piece 1: Root cap.

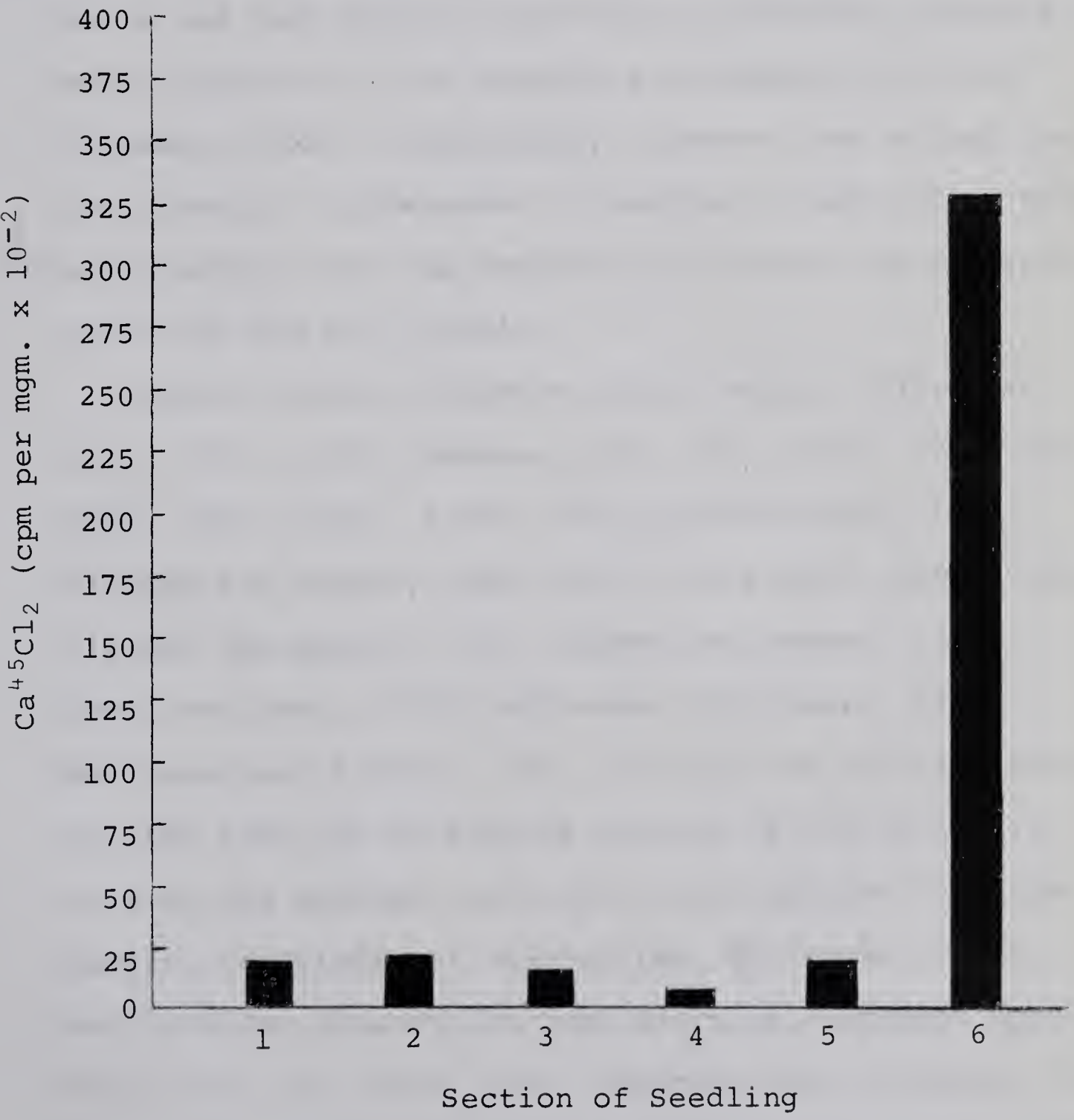
Piece 2: Meristematic and elongation region.

Piece 3: Cortex of the differentiation and hair region.

Piece 4: Stele of the differentiation and hair region.

Piece 5: Coleoptile of the shoot.

Piece 6: Primary leaves of the shoot.


400
375
350
325
300
275
250
225
200
175
150
125
100
75
50
25
0
$Ca^{45}Cl_2$ (cpm per mgm. x 10^{-2})
1
2
3
4
5
6
Section of Seedling

DISCUSSION

I. Epidermal Cell-wall and Root-hair Studies

"There is general agreement that root hairs result from retardation in vertical elongation of root epidermal cells and that they are produced by internal pressure on weaker portions of an unequally stiffened cell wall" (Cormack, 1962). Controversy, however, has arisen over the cause for differences in cell-wall composition which could account for the beginning of a hair and for apical growth of the hair itself.

Several writers (Roberts, 1916; McCoy, 1932; Farr, 1925, 1927, 1928; Cormack, 1935, 1944, 1945, 1949, 1954, 1955, 1956, 1959a, 1959b, 1961; Bennet-Clark, 1961; Cleland and Bonner, 1956; Cooil and Bonner, 1957; Ordin, Cleland and Bonner, 1957; Tagawa and Bonner, 1957; Deuel and Stutz, 1958; McClendon and Somers, 1960; Weintraub and Ragetli, 1961; Zaitlin and Coltrin, 1964) believe that the stiffening process is controlled in part by the gradual incorporation of calcium into the pectic materials of elongating epidermal cells and that the capacity to form hairs is dependent upon the rate at which this incorporation occurs. Other writers (Ekdahl, 1953, 1957; Belford and Preston, 1961) question the presence of bound calcium in the epidermal and root-hair walls. These writers are skeptical

concerning the stiffening of the growing cell wall by calcification and hold the view that the stiffening process is due entirely to changes in the cellulose component of the epidermal and root-hair cell walls.

Although calcification may not be the only process involved in the development of root hairs, the change of pectic materials to calcium pectate is known to occur in the walls of growing cells. According to Tupper-Carey and Priestley (1923), the pectic materials change from a protein-pectin complex in the walls of root meristematic cells to pectic acid in the walls of actively elongating cells and to calcium pectate in the walls of mature cells.

The observations of the present investigator not only confirm the earlier observations of Roberts (1916) and Cormack (1935) as to the presence of calcium in the epidermal and root-hair cell walls, but show that it occurs in the outer pectic layer of the wall quite distinct from the inner cellulose layer. Moreover, they are in exact agreement with the observations obtained in the earlier work with radioactive calcium (Cormack, Lemay and Maclachlan, 1962). However, because of refinements in earlier technique, it has been possible in the present investigation to study the actual calcification process in more detail. Furthermore, due to more careful washing methods of root material following treatment with Ca^{45}, the possibility that radioactivity demonstrated in the radio-

autographs could be due to the presence of calcium^{45} other than that incorporated in the cell wall and cytoplasm, could be definitely ruled out.

The calcification process as studied in the present investigation by means of radioautographs may be outlined as follows. The first indication of the incorporation of calcium^{45} into the elongating epidermal cell walls occurs in the region of the first-formed papillae. At the time of papillae formation, the transverse walls of an epidermal cell are already calcified. From here calcification progresses along the outer tangential wall but more rapidly along the basal portion of this wall than along the apical portion. Since calcium is believed to strengthen the growing cell wall, then the apical end of the cell which is least calcified would be the weakest. It is here that a papillae invariably pushes out (Roberts, 1916; Cormack, 1935, 1949). Furthermore, differences in the degree of calcification could account for differences in the rate of growth at the opposite ends of a papillae-forming cell. For instance, by the time the outer wall has been pushed out to form a papilla, the more strongly calcified basal end of the cell has stopped growing while the less calcified apical end continues to grow. By the time the papilla has grown into a short hair, the wall is uniformly calcified and vertical elongation of the hair-forming cell is arrested.

Evidence obtained in the present study shows that the same process of calcification that occurs in the middle lamella of an elongating hair-forming cell continues to occur uninterrupted behind the advancing tip of an actively-growing hair. The author believes that galacturonic acid which is the basis of pectin formation, is excreted at the tip of the hair, and that the changes to calcium pectate occur just back from the growing hair tip.

When roots are grown in a strong calcium^{45} solution, the hairs are stunted and show a heavy deposit of Ca^{45} over the entire dome of the root-hair tip. When these roots are transferred to a hypotonic solution, the hairs frequently burst at the tips. The bursting of the hairs at the tip under these conditions suggests that calcium^{45} is not bound in the root-tip wall as calcium pectate but exists in combination with substances other than pectic materials. (Possibly a protein-pectin complex similar to that described by Tupper-Carey and Priestley).

By means of refinements in technique it was possible to show in the present study that calcium^{45} is present in relatively large amounts in the cytoplasm some time before it appears in the epidermal cell walls as incorporated calcium^{45}. Calcium^{45} was detected along the plasma membranes as well as along the endoplasmic reticulum and around the nucleus during the early stages of

papilla formation and in the tips of actively growing hairs. This observation supports the view of Burström, 1954; Bennet-Clark, 1955; and Marinos, 1962; that calcium is associated with the general metabolism of the cell and with the semi-permeability (Von Guttenberg, 1951; Höfler, 1951) of the cytoplasmic membranes.

The main objection of Ekdahl, Belford and Preston to the calcification theory of root-hair development is that seedling roots of most plants produce abundant root hairs in moist air in the absence of any external calcium supply. That little calcium is necessary for the initiation of root hairs seems probable from results of the present study. Even in roots grown in contact with filter paper soaked with a calcium^{45} solution, the epidermal cell walls are only slightly calcified at the time of root-hair protuberance. In the case of roots grown in moist air but primarily germinated in Ca^{45} solution, the root hairs show almost no presence of calcium^{45} while the roots themselves are delicate and short-lived. It is known (Ordin, Cleland and Bonner, 1957) that in the absence of calcium the pectic acid is methylated to pectin.

The theory that gradual calcification is essential for the initiation of root hairs and for the normal growth of the hairs themselves has been greatly strengthened by the results of the present investigation.

The experiments with calcium^{45} can definitely show at what stage of epidermal cell development calcium^{45} becomes incorporated into the cell walls, that it increases gradually in amount during the formation of a papilla and increases further during the growth of a papilla into a hair.

II. Translocation and Distribution Studies of Calcium

The experiments with calcium^{45} introduced into the seed show that calcium is readily translocated from the root to the shoot. These results are in agreement with the conclusions of Crafts, 1949; and Bukovac and Wittwer, 1957. Ca^{45} introduced through the cotyledons of mustard seeds is observed to have slight translocation, but when introduced into the endosperm of corn, translocation was not detected. This difference in ability to translocate calcium was attributed to the deficiency of boron in the corn seed. To test the validity of this assumption, boric acid was introduced with Ca^{45} into a hole bored in the endosperm of the corn seed. Under this condition, small amounts of Ca^{45} was translocated down the root and up the shoot. Although a functional relationship between calcium and boron has been established, the mechanism of this relationship is not clearly understood (Smith, 1944; Shive, 1945; Marinos, 1962; Brewbacker and Kwack, 1963).

The results of experiments with corn and mustard

seedlings grown with only the root tip, root hairs, and the seed in contact with the Ca^{45} moistened filter paper, show that calcium^{45} translocates through the root hair, through the epidermal cells and across the cortical cells in a narrow radial band to the nearest xylem elements. Calcium translocation through the cortex is not diffuse but rather it is confined to a narrow pathway.

In mustard roots, calcium^{45} was shown to accumulate at the base of a hair-forming cell, in several adjacent cortical cells and in the large intercellular spaces. The accumulation of calcium at the epidermal hair-forming cell is in agreement with Hylmö, 1953; and Skelding and Rees, 1952; who have found that in phase two of salt transport there is an accumulation of salts in the vacuole which is metabolically maintained. The author concludes that the accumulation of calcium^{45} in the outer cortical region opposite actively growing hairs facilitates downward translocation in the intercellular spaces to the root apex. The belief that the calcium^{45} translocates down the root through the intercellular spaces was largely concluded from the observation that calcium^{45} is heavily concentrated in the intercellular spaces at the level of the root immediately below the root-hair region, and diminishes gradually from this point downwards to the root apex. Non-calcified root

caps and the observation that calcium^{45} accumulates in the intercellular spaces, strengthens the view (Kramer, 1945) that calcium^{45} enters the root through the root hairs and not through the root tip.

According to the experiments of Sorokin, 1958, and Peterson, 1964, the intercellular spaces in the region of elongation contain a highly complex substance referred to as the "intercellular tubular matter", while in the region of mature root hairs the spaces are empty. The presence or absence of calcium^{45} in the intercellular spaces at different levels of the seedling root could be related to the presence or absence of the intercellular tubular matter.

That calcium necessary for cell wall formation translocates from the intercellular spaces to the short epidermal cells was postulated by Cormack, 1948. Experiments with radioactive leucine by Cormack and Lemay in 1963 have definitely shown that substances translocate through the spaces. The results of the present investigation confirm that Ca^{45} translocates through the spaces to the short cells. Ca^{45} is observed to be much more concentrated in the hair-forming short cells than in the hairless long cells. The difference in the amount of accumulated calcium^{45} between the short and the long epidermal cells may be attributed to differences in the position of these cells to radiating rows of intercellular spaces

and to differences in acidity of the cell sap.

The results of experiments with seedlings labelled by the root-tip immersion method confirm the conclusions of Von Guttenberg, 1943; and Kramer, 1945; that calcium is absorbed over the whole surface of the actively growing root except in the region covered by the root cap.

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